RESPIRATION OF THE SPADIX FROM THE AROID *ZANTEDESCHIA AETHIOPICA* SPRENG.

By M. D. HATCH* and ADELE MILLERD†

[*Manuscript received December* 10, 1956]

Summary

Spadix tissue of a number of aroid species has been shown to be an outstanding exception to the general pattern of plant respiration. In the work reported here the respiration of whole tissue and mitochondria from the spadix of another aroid species *Zantedeschia aethiopica* Spreng. was studied. The respiration of both whole tissue and mitochondria was found to be strongly inhibited by the metal-complexing agents cyanide and azide. Cytochrome oxidase and cytochromes *b* and *c* were spectroscopically indentified in mitochondrial suspensions and cytochrome oxidase activity was demonstrated in extracts. The results indicate that the respiratory characteristics of the *Zantedeschia* spadix tissue are similar to those of most other plant tissues. Such characteristics are, however, in marked contrast to the respiratory pattern reported for other aroid spadices.

I. INTRODUCTION

It is apparent that aroid spadix tissue provides an outstanding exception to the general pattern of plant respiration. This tissue is characterized by an exceptionally rapid respiration rate (500–3000 μl O_2/g fresh wt./hr). In 10 species reported (van Herk 1937; James and Beevers 1950; van Norman 1955; Yocum and Hackett 1955) this respiration was unaffected or only slightly inhibited by cyanide even at a concentration as high as 10^{-2}M. In contrast to cytochrome oxidase-mediated respiration, the respiration rate of these tissues is apparently directly dependent on oxygen tensions up to 100 per cent. Such exceptions to the usual respiratory pattern of plants are of considerable importance at a time when there is a growing realization of the marked overall uniformity of fundamental biochemical pathways.

This paper is concerned with the respiratory properties of the whole tissue and mitochondria of the spadix of the aroid *Zantedeschia aethiopica* Spreng. (arum lily). The respiratory characteristics of this tissue are discussed in relation to those of other aroid species, and to the general pattern of plant respiration.

II. MATERIALS

Spadix tissue of *Z. aethiopica* was used. Material was chosen at a stage of maturity equivalent to the δ–ϵ stage of the James and Beevers (1950) development classification. The spadix is differentiated into a central rachis, about which are compactly arranged the yellow male flower structures. The respiratory properties of both the whole spadix and these separate portions were studied.

* Plant Physiology Unit, Division of Food Preservation and Transport, C.S.I.R.O., and Botany School, University of Sydney.

† Department of Biochemistry, University of Sydney.

III. Methods

(a) *Preparation of Tissue Slices*

Slices (approx. 1 mm thick) were cut from the whole spadix and washed with three changes of distilled water. The randomized slices were blotted dry on filter paper, weighed into equal lots (1 g ≡ 125 mg dry weight), and added to manometer flasks. To determine the error of such sampling the respiration rates of five replicate experiments were examined. The slices were suspended in 0·066M phosphate buffer (pH 5·0). The standard deviation calculated was 1·5 per cent. of the arithmetic mean. In experiments in which the differentiated portions of the spadix were studied, male flower segments (0·5 g ≡ 0·076 mg dry weight), which were easily removed from the rachis, were used as such after an initial washing with distilled water. Slices of the rachis (0·5 g ≡ 0·058 mg dry weight) were prepared in a similar manner to the whole spadix slices.

(b) *Preparation of Cytoplasmic Particles*

Whole spadix tissue (15 g) or its differentiated parts were washed in distilled water, sliced, then ground in a mortar with sand and 50 ml of 0·5M sucrose. The homogenate was strained through muslin, and the filtrate centrifuged for 3 min at 2000 *g*. The supernatant was then centrifuged at 16,000 *g* for 10 min. The residue obtained was washed twice by resuspension in 0·5M sucrose and recentrifugation at 16,000 *g*. The final residue was suspended in 0·5M sucrose. Aliquots (0·4–0·6 mg total nitrogen) of this suspension were used in manometric experiments. All operations were carried out between 0 and 2°C. The addition of "Versene" to the preparation media had no detectable effect on the activity of the isolated particles.

(c) *Application of Inhibitors*

Mixtures of calcium hydroxide and calcium cyanide (Robbie and Leinfelder 1945; Robbie 1948) were used to absorb carbon dioxide in the experiments on the effects of cyanide on respiration. When azide was used as an inhibitor, the tissue was allowed to incubate with the inhibitor for 30 min before potassium hydroxide was added to the centre well. This was done to minimize the effects of the diffusion of azide into the alkali of the centre well.

Oxygen uptake was measured at 30°C by the standard Warburg manometric technique. Spectroscopic examination of mitochondrial suspensions was carried out with a Zeiss-Winkel microspectroscope equipped with a wavelength scale. Sodium hydrosulphite was used as a reducing agent. Total nitrogen was determined by Nesslerization of acid digests of mitochondrial suspensions. The coenzyme concentrate was prepared from dried brewer's yeast (Le Page and Mueller 1949). Cytochrome *c* was prepared by the method of Keilin and Hartree (1937) and standardized spectrophotometrically (Umbreit, Burris, and Stauffer 1951). Cytochrome oxidase activity was assayed according to Umbreit *et al.* (1951).

IV. Experimental

(a) *Respiration of Sliced Tissue*

The respiration of spadix tissue slices from all aroids so far examined has been remarkably resistant to cyanide, and, where tested, to azide (van Herk 1937; James

and Beevers 1950; James 1953; van Norman 1955). It is of interest therefore that slices from the spadix of *Z. aethiopica* show a quite different response to these inhibitors. Cyanide (10^{-3}M) caused 55 per cent. inhibiton during the first hour and 89 per cent. during the third hour (Fig. 1). Azide (10^{-3}M) was an even more rapidly effective inhibitor resulting in an 83 per cent. inhibition during the first hour and an 87 per cent. inhibition during the third hour (Fig. 1).

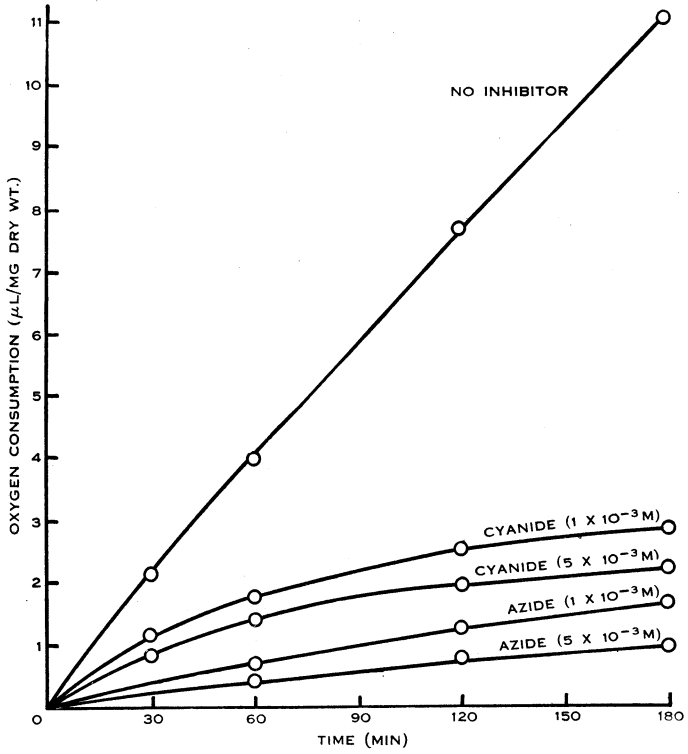

Fig. 1.—Effect of cyanide and azide on the respiration of *Zantedeschia* spadix slices. The control system contained tissue (1·0 g wet wt.) suspended in 0·06M phosphate buffer at pH 5·0.

The pH at which these ionic inhibitors are applied to plant tissue is an important factor in determining the rate at which they penetrate the tissue, and therefore the degree of inhibition observed (James 1953). In a separate experiment it was shown that the respiration of *Zantedeschia* spadix was only slightly inhibited after 2 hr by cyanide and azide (5×10^{-3}M) at pH 7·0. At pH 5·0, however, azide exerts its inhibitory effect rapidly, but the effect of cyanide, although considerably more rapid than at pH 7·0, is still apparently limited during the first hour by its rate of penetration to the site of inhibition (Fig. 1).

A respiratory rate of 500 μl O_2/g fresh wt./hr was recorded for *Zantedeschia* spadix tissue. Although this is not as high as the rates observed in some other spadix tissue (Yocum and Hackett 1955), it is, nevertheless, high compared with most plant tissues.

The spadix tissue, as is shown in Figure 1, had a basal respiration stable to cyanide. The respiration of both the male flower segments and the rachis contributed to this cyanide-stable portion of the respiration of the whole tissue. However, as is shown in Figure 2, the male flower portions had a somewhat larger cyanide-stable basal respiration (65 per cent. inhibition with 5×10^{-3}M cyanide after 2 hr) than did the rachis (79 per cent. inhibition with 5×10^{-3}M cyanide after 2 hr). The effect of azide on the respiratory rates of both the rachis and male flower portions

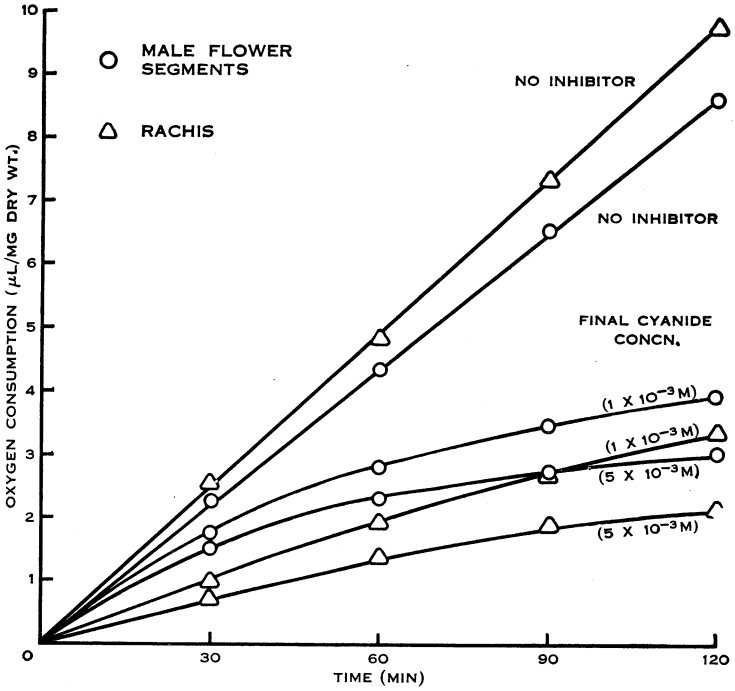

Fig. 2.—Effect of cyanide on the respiration of the differentiated portions of the *Zantedeschia* spadix. The control system contained tissue (0·5 g wet wt.) suspended in 0·06M phosphate buffer at pH 5·0. The final cyanide concentrations are as indicated.

was similar to that of cyanide. Azide (10^{-3}M), however, was a somewhat more efficient inhibitor than cyanide, causing, after 2 hr, 91 per cent. inhibition of rachis respiration, and 80 per cent. inhibition of the respiration of the male flower segments.

(*b*) *Oxidative Activity of Isolated Mitochondria*

Since it is well established that the enzymes responsible for the aerobic phases of respiration are localized in the mitochondria, it was of interest to examine mitochondria isolated from *Zantedeschia* spadix. Such cytoplasmic particles rapidly oxidized all the acids of the Krebs cycle (Table 1). Oxidation of pyruvate was dependent upon the concomitant oxidation of a small amount of one of the other acids of the cycle (Table 2). These findings are in agreement with Hackett and Simon (1954) for mitochondria isolated from *Arum* spadix.

The results shown in Tables 1 and 2 represent the activities of mitochondria isolated from the whole spadix tissue. Table 3 gives details of the oxidative activity

TABLE 1

OXIDATION OF KREBS-CYCLE ACIDS BY MITOCHONDRIA ISOLATED FROM ZANTEDESCHIA SPADIX

The complete system contained mitochondrial suspension; substrate (2×10^{-2}M); phosphate buffer pH 7·0 ($2{\cdot}5\times10^{-3}$M); sucrose (5×10^{-1}M); $MgSO_4$ (1×10^{-3}M); adenosine monophosphate (1×10^{-3}M); yeast nucleotide (1 mg). Total volume 2·0 ml

Experiment	Substrate	Oxygen Uptake (μl O_2/mg N/30 min)
1	None	27
	Succinate	226
	α-Ketoglutarate	102
2	None	22
	Citrate	170
	Malate	92

of mitochondria isolated from the two portions of the spadix, the rachis and the male flower segments. Mitochondria isolated from the rachis were the more active on a

TABLE 2

PYRUVATE OXIDATION BY ZANTEDESCHIA SPADIX MITOCHONDRIA CATALYSED BY KREBS-CYCLE ACIDS

Complete system as in Table 1. Substrate concentration as indicated

Experiment	Substrate	Concn. (M)	Oxygen Uptake (μl O_2/mg N/30 min)	
			Krebs-cycle Acid	Krebs-cycle Acid +0·02M Pyruvate
1	None		23	—
	Pyruvate	2×10^{-2}	24	—
	Succinate	1×10^{-3}	73	125
	Malate	1×10^{-3}	48	77
2	None		21	—
	Pyruvate	2×10^{-2}	24	—
	α-Ketoglutarate	1×10^{-3}	37	86
	Citrate	1×10^{-3}	36	74

Q_{O_2} (N) basis but those from the male flower segments were responsible for most of the endogenous oxygen consumption. When mitochondria were prepared from

whole tissue the endogenous oxygen consumption was not reduced by repeated washing of the isolated particles and was completely insensitive to cyanide (Table 4). The results recorded in Table 3 indicate that the mitochondria from the male flower

TABLE 3

OXIDATIONS OF KREBS-CYCLE ACIDS BY MITOCHONDRIA ISOLATED FROM THE DIFFERENTIATED PARTS OF ZANTEDESCHIA SPADIX

Complete system as in Table 1

Substrate	Oxygen Uptake (μl O_2/mg N/30 min)	
	Male Flower Segments	Central Rachis Tissue
None	29	11
Succinate	145	409
α-Ketoglutarate	80	152
Citrate	93	258
Malate	80	134

segments were responsible for most of this cyanide-stable endogenous oxygen consumption. Such an observation is in keeping with the results expressed in

TABLE 4

INHIBITION OF MITOCHONDRIAL OXIDATIONS BY CYANIDE: WHOLE SPADIX

Complete system as in Table 1

Experiment	Substrate	Oxygen Uptake (μl O_2/mg N/30 min)	
		Krebs-cycle Acid	Krebs-cycle Acid + 0·001M Cyanide
1	None	16	18
	Succinate	130	14
	α-Ketoglutarate	74	16
2	None	22	22
	Citrate	84	20
	Malate	67	24

Figure 2. Although the endogenous oxygen consumption of the isolated mitochondria was cyanide-stable, oxidation of intermediates of the tricarboxylic acid cycle was completely inhibited by 1×10^{-3}M cyanide (Table 4).

(c) *Demonstration of the Complete Cytochrome System in Mitochondria Isolated from* Zantedeschia *Spadix*

The cyanide sensitivity of mitochondrial oxidations (Table 4) suggests that the cytochrome system may well be operating in these particles. This has been shown by assaying the cytochrome oxidase activity of these particles and by spectroscopic identification of the three cytochromes.

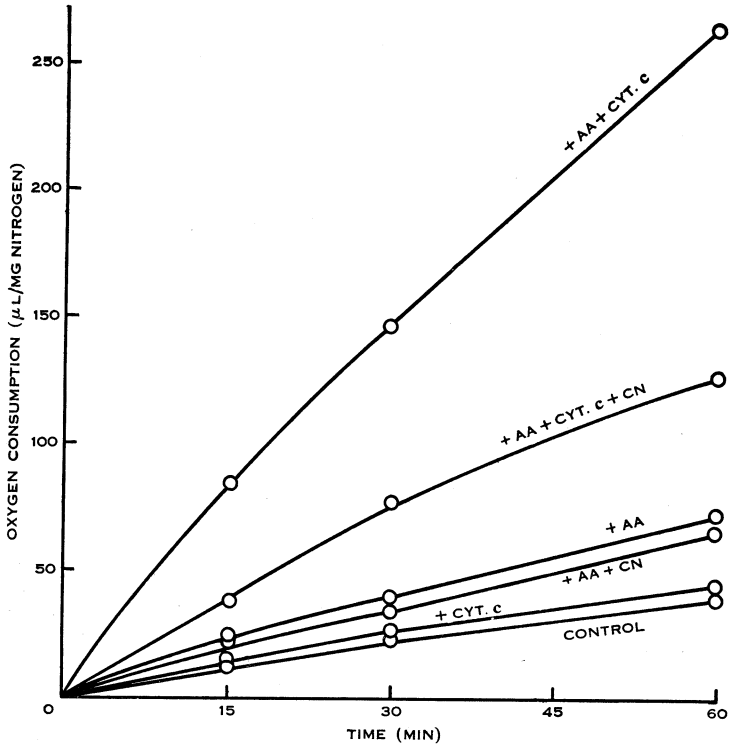

Fig. 3.—Cytochrome oxidase activity in a particulate preparation from *Zantedeschia* spadix mitochondria. The control system contained particulate enzyme (0·58 mg nitrogen), 0·025M phosphate buffer at pH 7·0, and 0·001M $MgSO_4$. Additions were: Ascorbic acid (AA) (1×10^{-2}M), cytochrome *c* (CYT. *c*) (3×10^{-5}M), and hydrogen cyanide (CN) ($2{\cdot}5 \times 10^{-4}$M). Total volume 2 ml.

(i) *Cytochrome Oxidase Activity of* Zantedeschia *Mitochondria.*—Mitochondria were prepared as described in Section III to the first washing stage, but the particles were then washed twice with distilled water. This procedure damages the structure of the mitochondria and, if cytochrome oxidase is operating, the particles will then show a requirement for cytochrome *c*. The oxidation of ascorbic acid by these particles was then examined, and, as is shown in Figure 3, this oxidation was markedly stimulated by the addition of cytochrome *c*. This cytochrome-mediated oxidation of ascorbic acid was cyanide-sensitive (60 per cent. inhibition at a cyanide concentration of $2{\cdot}5 \times 10^{-4}$M).

(ii) *Spectroscopic Identification of the Cytochromes in Mitochondrial Suspensions.*– No absorption bands were observed in the untreated mitochondrial suspensions. When sodium hydrosulphite was added as a reducing agent three strong absorption bands appeared at 604, 563, and 553 mμ. These bands correspond to the α-absorption bands of the reduced forms of cytochrome oxidase and cytochrome *b* and *c* components respectively.

V. Discussion

Plant tissues may vary considerably in respiratory rate. Variations from 10 to 500 μl O_2/g fresh wt./hr are normally encountered. The total respiration of most plant tissues can be divided into two components defined by their behaviour to such metal-complexing agents as cyanide and azide. The component insensitive to such inhibitors is termed the "basal" respiration. This component characteristically contributes to only a small portion of the total respiration, and its magnitude is usually unaffected during changes in total respiration of a tissue. The major portion of the total respiration, which may be termed "non-basal", is inhibited by cyanide and may be extensively varied by different tissue treatments. The terminal oxidase system mediating this respiration has been identified in many tissues as the classical cytochrome system situated in the mitochondria.

The respiration of the spadix of a number of aroid species (van Herk 1937; James and Beevers 1950; van Norman 1955; Yocum and Hackett 1955) provides an outstanding exception to this pattern of plant respiration. In all species examined the respiration rate was shown to be exceptionally rapid in comparison with other plant tissues. This rapid respiration was apparently quite stable to such metal-complexing agents as cyanide and azide. In addition the respiration of tissue slices and homogenates from *Arum maculatum* L. was shown to be directly dependent upon oxygen tensions up to 100 per cent. (James and Beevers 1950). This is in sharp contrast to cytochrome-mediated respiration. Such observations have led to the general acceptance of a non-metallic oxidase as the functional terminal oxidase in these tissues. The behaviour of aroid spadix tissue to oxygen tensions has been further examined in *Peltandra* and a tropical *Philodendron* (Yocum and Hackett 1955). Sensitivity to oxygen tension was shown by these tissues only when the respiration was examined in a liquid phase. In air, the tissue slices showed a high affinity for oxygen and a low but measurable affinity for carbon monoxide. Such a combination of properties is, as the authors point out, not typical of any known non-metallic oxidase.

It is well established that the enzymes responsible for the aerobic phases of respiration are localized in the mitochondria, and it was thus of interest to examine the properties of mitochondria isolated from these aroids. Mitochondria which rapidly oxidized Krebs-cycle intermediates were readily isolated from the spadix of *A. maculatum* (Hackett and Simon 1954). Such oxidations were not inhibited by cyanide (James and Elliott 1955). Mitochondria were also isolated from the spadix of *Symplocarpus foetidus* Nutt. These particles rapidly oxidized Krebs-cycle acids and the absorption maxima of cytochrome *a*, *b*, and *c* components were spectroscopically identified (Bonner and Yocum 1956; Hackett 1956). The effects of cyanide and

azide on these mitochondrial oxidations were not typical of an oxidase system mediated by cytochrome oxidase alone. However, the cytochrome oxidase activity of these particles was itself sensitive to both inhibitors.

Bonner and Yocum (1956) indicated that the results were consistent with the view that the cytochrome *b* component which absorbs at 560 mμ is capable of linking dehydrogenases not only to cytochrome *c* but also directly to molecular oxygen. More recently, supporting evidence for this conclusion was obtained by Bendall and Hill (1956). They showed that tissue slices and mitochondrial suspensions prepared from *A. maculatum* contained a full complement of cytochromes in relatively high concentrations. In highest concentration was the cytochrome *b* component which absorbed at 560 mμ and was rapidly oxidized by molecular oxygen in the presence of cyanide. Although a normal cytochrome oxidase is present in these tissues it appears that the passage of electrons from substrate to molecular oxygen is, in part at least, diverted through this autoxidizable cytochrome *b* component in the intact tissue.

It is apparent then that spadix tissue from the aroids so far examined display respiratory behaviour quite atypical of most plant tissues.

The spadix of *Z. aethiopica*, however, presents a very different picture. Tissue slices from the spadix of this aroid, as from other aroids investigated, respired at a rapid rate (500 μl O_2/g fresh wt./hr), but *Zantedeschia* spadix respiration was quite sensitive to cyanide and azide (Fig. 1) provided that the inhibitor was applied at a sufficiently acid pH. Experiments using ionic inhibitors are significant only if the relationship between pH and penetration of the inhibitor has been considered. The respiration of the differentiated portions of the spadix was separately examined (Fig. 2). It was apparent that in the male flower segments the cyanide-stable basal respiration represented a greater portion of the total respiration than it did in the rachis.

Mitochondria isolated from *Zantedeschia* spadix rapidly oxidized tricarboxylic acid intermediates (Tables 1 and 2). Such oxidations were inhibited by cyanide (Table 4). Cytochrome oxidase activity was high in these particles (Fig. 3) and the typical α-absorption bands of cytochrome oxidase and cytochromes *b* and *c* were demonstrated. These mitochondria then show the general characteristics of mitochondria isolated from many plant sources but are different from those isolated from other aroid spadices.

Zantedeschia spadix therefore shows a normal respiratory pattern, and cytochrome oxidase is apparently the major terminal oxidase. The small component (10–15 per cent.) of the total respiration which is insensitive to cyanide and azide corresponds to the basal respiration characteristic of most plant tissues. Thus, although the respiration of *Zantedeschia* spadix is similar to that of the majority of plant tissues it is in marked contrast with that of spadices from other aroid species.

The possibility arises that there may be a correlation between the morphology and respiratory properties of these various spadix tissues. Of the types dealt with by James and Beevers (1950) (*Arum*, *Arisaema*, *Biarum*, *Amorphophallus*, and *Sauromatum*) each spadix has a barren terminal appendage (appendix) which was the portion

used as the experimental material. The basal portion of the spadix which bears bands of unisexual male and female flowers was not studied.

None of the other types which showed a respiration characteristic of the aroids described by James and Beevers (1950) has a similar spadix morphology. The spadix of *Symplocarpus foetidus* is completely covered with hermaphrodite flowers. *Peltandra* and *Philodendron* spadices bear unisexual flowers covering the whole surface, with the female flowers at the base and the male flowers on the upper portion.

The arrangement of flowers on the spadix of *Z. aethiopica* closely resembles that of *Peltandra* and *Philodendron*. The upper four-fifths of the total spadix length is covered by male flowers and the remainder by female flowers.

It is apparent that no obvious correlation exists between the morphology and the respiratory properties of the spadix tissues studied. The general morphology of the cyanide-insensitive types is not uniform nor are there significant differences between this group and *Z. aethiopica* which has a normal cyanide-sensitive respiration.

VI. Acknowledgments

This work was carried out partly in the Department of Biochemistry, University of Sydney, and partly in the Plant Physiology Unit of the Botany School, University of Sydney, and of the Division of Food Preservation and Transport, C.S.I.R.O. It was supported by a University of Sydney Research Grant.

The authors wish to express their thanks to Dr. R. N. Robertson, Dr. F. E. Huelin, and Mr. K. T. Glasziou for helpful criticism of the manuscript.

VII. References

Bendall, D. S., and Hill R. (1956).—*New Phytol.* **55**: 206.
Bonner, W. D., and Yocum, C. S. (1956).—*Plant Physiol.* **31** (suppl.): xli.
Hackett, D. P. (1956).—*Plant Physiol.* **31** (suppl.): xl.
Hackett, D. P., and Simon, E. W. (1954).—*Nature* **173**: 162.
van Herk, A. W. H. (1937).—*Rec. Trav. Bot. Néerl.* **34**: 69.
James, W. O. (1953).—*Annu. Rev. Pl. Physiol.* **4**: 59.
James, W. O., and Beevers, H. (1950).—*New Phytol.* **49**: 353.
James, W. O., and Elliott, D. C. (1955).—*Nature* **175**: 89.
Keilin, D., and Hartree, E. F. (1937).—*Proc. Roy. Soc.* B **122**: 298.
Le Page, G. A., and Mueller, G. C. (1949).—*J. Biol. Chem.* **180**: 975.
van Norman, R. W. (1955).—*Plant Physiol.* **30** (suppl.): xxix.
Robbie, W. A. (1948).—"Methods in Medical Research." Vol. 1. p. 307. (Year Book Publ. Co.: Chicago.)
Robbie, W. A., and Leinfelder, P. J. (1945).—*J. Industr. Hyg.* **27**: 269.
Umbreit, W. W., Burris, R. H., and Stauffer, J. F. (1951).—"Manometric Techniques and Tissue Metabolism." (Burgess Publ. Co.: Minneapolis.)
Yocum, C. S., and Hackett, D. P. (1955).—*Plant Physiol.* **30** (suppl.): xxx.