BBA 45988

CYANIDE-INDUCED TRANSITION FROM ENDOGENOUS CARBOHYDRATE TO LIPID OXIDATION AS INDICATED BY THE CARBON-13 CONTENT OF RESPIRATORY CO_2

BRUCE S. JACOBSON *, GEORGE G. LATIES *, BRUCE N. SMITH, SAMUEL EPSTEIN AND BETSY LATIES *

* *Department of Botanical Sciences and Molecular Biology Institute, University of California, Los Angeles, Calif. 90024 and the Division of Geological Sciences, California Institute of Technology, Pasadena, Calif. 91109 (U.S.A.)*

(Received March 23rd, 1970)

SUMMARY

The respiration of thin aerated discs of potato tuber tissue rises sigmoidally through 24 h. Aged disc respiration is ostensibly resistant to concentrations of cyanide which inhibit the respiration of fresh discs. It has been shown that cyanide-resistant respiration does not represent indifference to the inhibitor, but is rather due to the suppression of one respiratory carbon path and the evocation of another. The predominant respiratory carbon path of aged discs in the absence of cyanide comprises glycolysis linked to the tricarboxylic acid cycle.The carbon path mediating the cyanide-induced respiration reflects tricarboxylic acid cycle-independent lipid degradation.

The respiratory substrate at any time was deduced by comparing the $^{13}C/^{12}C$ ratio of respired CO_2, collected from discs in the presence or absence of cyanide, with the $^{13}C/^{12}C$ ratios characterizing endogenous potential metabolites. The determination of the predominant respiratory substrate in potato discs, which have an endogenous substrate reserve, proved possible because the relative concentrations of the stable carbon isotopes in endogenous compounds such as lipid and starch are widely different.

INTRODUCTION

The respiration of a variety of plants and animals is frequently ostensibly resistant to cyanide, a ligand complexing agent of cytochrome oxidase. Complete or partial cyanide resistance may be a natural condition of the tissue, or it may be induced. A classic example of natural resistance is the cyanide-insensitive respiration of the Arum spadix[1]. Induced resistance may result from experimental alteration. For example, the respiration of freshly sliced storage tissue is sensitive to cyanide, while that of aged tissue is frequently insensitive[2,3]. Whether cyanide resistance is natural or evoked, respiration in the presence of cyanide is apparently non-phosphorylative[4,5]. Further, while the gross respiration may appear indifferent to cyanide, there is evidence that resistance is illusory; that is, a cyanide-resistant respiratory

path may be evoked by cyanide and obscure cyanide inhibition of normal respiratory electron flow[5–7].

It has been postulated that cyanide resistance is due to electrons by-passing cytochrome oxidase by way of a *b*-type cytochrome[4,8]. Such a by-pass was suggested by the observation that mitochondria from Arum spadices or skunk cabbage contain a high concentration of an autoxidizable cytochrome *b* that remains oxidized in the presence of cyanide[9–11]. While the above view imputes resistance solely to a change in the path of electron transport, and tacitly presumes an unaltered respiratory carbon path, essentially nothing is in fact known of the carbon path in cyanide-treated tissue, and studies of isolated mitochondria in relation to the cyanide response may, by their very nature, beg the question of the carbon path in intact tissue.

A carbon path different from the normal respiratory pathway and linked to a cyanide-resistant terminal oxidase was suggested from experiments with aged potato tuber tissue[6,7]. By presenting labeled glucose and determining the influence of cyanide on both $^{14}CO_2$ evolution and on the labeling pattern of tricarboxylic acid cycle intermediates, it was concluded that cyanide inhibits glucose oxidation while at the same time evoking another, yet unknown, carbon path[7]. The cyanide elicited metabolism was termed compensatory respiration, since O_2 uptake and CO_2 evolution continued unabated while the normal oxidative pathway was inhibited[7]. Whereas the foregoing type of experiments permits the recognition of compensatory respiration, such experiments fail to elucidate the substrate or the nature of the compensatory pathway.

Results to be presented demonstrate that while starch is normally the predominant respiratory substrate in aged potato discs, cyanide concurrently represses starch metabolism and stimulates lipid oxidation. The mechanism of lipid degradation educed by cyanide is other than β-oxidation linked to tricarboxylic acid cycle metabolism.

Experimental rationale. As with most storage tissues, the respiration of potato tuber slices does not depend upon exogenous carbon sources. In consequence, the oxidation of exogenously provided labeled compounds throws no light on the endogenous respiratory carbon path. However, an analysis which takes advantage of the differing relative concentrations of the stable carbon isotopes in major classes of endogenous metabolites can be used to determine the substrate for cyanide-induced compensatory respiration.

The relative concentrations of carbon-13 and carbon-12, *i.e.*, the $^{13}C/^{12}C$ ratios, in various cellular constituents (*e.g.* lipids, starch, *etc.*) are different[12–14]. If the differences between the $^{13}C/^{12}C$ ratios of the various constituents are sufficiently large, the endogenous respiratory substrate may be determined by comparing the $^{13}C/^{12}C$ ratio of respired CO_2 with the stable carbon isotope ratios of the major classes of metabolites[14]. The concept that the $^{13}C/^{12}C$ ratio of respired CO_2 reflects the ratio in the substrate being metabolized has been verified, and used to establish a change in respiratory substrate from lipid to starch during the aging of potato tuber discs[14,15].

The means used to deduce the substrate for cyanide-resistant respiration was, therefore, to determine $^{13}C/^{12}C$ ratio of respiratory CO_2 in the presence of cyanide, and to compare the ratio with that characterizing potential substrates. When the type of endogenous substrate in the presence of cyanide was once determined, further

experiments with carbon-labeled substrates elucidated more fully the nature of the cyanide-resistant carbon path.

MATERIALS AND METHODS

Tissue discs 1 mm thick and 10 mm in diameter were prepared from Russet Burbank potatoes as previously described[14]. The discs were aerated in 0.1 mM $CaSO_4$ for 20 h, at which time respiration appears cyanide resistant[3]. The solution, maintained at pH 5.0, was changed 4 times during the aging period. Respiratory CO_2 was collected from aged discs for the determination of $^{13}C/^{12}C$ ratios. Experiments with carbon-labeled substrates were carried out with the same tissue.

CO_2 collection

The system for collecting respired CO_2 has been described[14,16]. Fifty g of potato discs were placed in a 1-l flask containing 200 ml of 0.1 mM $CaSO_4$. The pH of the solution was monitored and kept at 5.0 with a pH-stat (0.2 M H_2SO_4 as titrant). Following a preincubation period in a stream of CO_2-free air, the flask was sealed and gently incubated on a rotary shaker for 40 min. Subsequently CO_2-free air was drawn through the system for an additional 20 min, the CO_2 collected being that evolved in 1 h. When CO_2 was collected in the presence of cyanide, the above procedure was used with cyanide added after the first hour of incubation and collection. The initial concentration of HCN was 0.22 mM. CO_2 was collected in traps held in liquid nitrogen[16]. A trap in dry ice and acetone between the incubation flask and the liquid nitrogen trap removed water and cyanide.

Mass spectrometer analysis

$^{13}C/^{12}C$ ratios (± 0.01 %) are determined and reported relative to the $^{13}C/^{12}C$ ratio of a standard. The standard used is the CO_2 from the fossil carbonate skeleton of *Belemnitella americana* (PDB_1). The function defining the reported values is

$$\delta^{13}C \text{ per mil} = \frac{^{13}C/^{12}C \text{ sample} - ^{13}C/^{12}C \text{ standard}}{^{13}C/^{12}C \text{ standard}} \times 10^3$$

Since the standard has a relatively large $^{13}C/^{12}C$ ratio, all δ values reported in this article are negative. The point to emphasize is that the δ value of the respired CO_2 reflects the δ value of the metabolized substrate.

Potential respiratory substrates were isolated by a variety of procedures[14]. Extracted metabolites were combusted to CO_2 at 800° in an evacuated closed system containing oxygen[17]. The $\delta^{13}C$ values of the CO_2 samples were determined with a Nier sixty-degree sector-type mass spectrometer as modified by McKinney *et al.* (see ref. 16). The latter instrument allows the accurate determination of the $^{13}C/^{12}C$ ratio of a sample by simultaneously collecting both isotopic species and electronically equalizing the small ^{13}C and large ^{12}C signals. When the $^{13}C/^{12}C$ ratio of the standard is set as a frame of reference, the ratio of the sample can be compared, and ascertained with great precision.

Label experiments

Forty-five discs (about 3 g fresh weight) were incubated in 15 ml of experimental solution in a 125-ml erlenmeyer flask. Solutions were 25 mM potassium biphthalate

(pH 5.0) except in experiments involving malonate, where 50 mM potassium malonate (pH 5.0) served as buffer as well. Where cyanide was present the concentration was 0.3 mM. Labeled compounds were added as follows: 5 μC [2,3-$^{14}C_2$]succinate (9.8 μC/μmole), 10 μC [1-^{14}C]myristic acid (4 μC/μmole), or 10 μC uniformly ^{14}C-labeled glucose (4 μC/μmole). Samples were incubated in a water bath rotary shaker at 25°. Respiratory CO_2 containing $^{14}CO_2$ was absorbed in 0.18 ml 10 % KOH dispersed on a 1 × 8 cm strip of Whatman GF/A glass paper bent into a loop and suspended from a hook fixed into the center of a rubber stopper tightly held in the top of the erlenmeyer flask. Tissue was incubated for 80 min. The KOH loops were changed every 20 min. The dried loops were added directly to a vial with 15 ml of toluene containing per l 4 g 2,5-diphenyloxazole and 0.05 g *p*-phenylbis-(5-phenyloxazole). Radioactivity was determined with a scintillation counter.

RESULTS

Effect of cyanide on fresh and aged disc respiration

Whereas the respiratory rate of potato discs increases fourfold during 24 h of aging, the sensitivity to cyanide decreases. Thus the rate of oxygen uptake by aged discs is but slightly inhibited by a range of cyanide concentrations from 0.1 to 0.5 mM, while the inhibition of fresh discs is considerable, and attains a maximum at 0.3 mM HCN (Fig. 1). When discs are incubated in 1 mM HCN for 90 min, fresh discs take up about 30 % more cyanide than aged discs, as measured by the loss of cyanide from the incubation medium. However, the greater sensitivity of fresh disc respiration is not attributable to the greater rate of HCN uptake, since even quadrupling the lowest cyanide concentration which evokes maximum inhibition in fresh discs fails to significantly inhibit aged disc respiration (see Fig. 1).

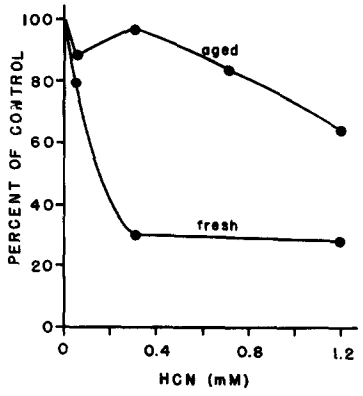

Fig. 1. Effect of HCN on the respiration of fresh and aged potato tuber discs. The amount of O_2 taken up by the tissue incubated at 25° was measured manometrically. Readings were made every 30 min for a total time of 1.5 h. The tissue was incubated in HCN for 30 min prior to the first reading. Each point represents an average of two experiments.

Endogenous respiratory substrate as determined by carbon isotope ratios

The δ values of potential respiratory substrates are given in Table I. The δ value of lipid is more negative than the values for starch or protein; *i.e.* starch and protein have greater $^{13}C/^{12}C$ ratios than does lipid. The smaller $^{13}C/^{12}C$ ratio of lipid

TABLE I

$\delta^{13}C$ PER mil OF PRIMARY ENDOGENOUS SUBSTRATES FROM POTATO TUBER TISSUE

Substrate	$\delta^{13}C$ *per mil* *
Lipid	-34.84 ± 0.330
Protein	-26.64 ± 0.155
Starch	-25.51 ± 0.208

* Average of four determinations $\pm$ S.E.

relative to that of carbohydrate has been observed in all organisms studied[12,13]. It is interesting that the difference between the two ratios is greatest in organisms with a low lipid content[13].

The δ value of CO_2 collected from potato discs aged 20–24 h is about -26 per mil. That is, the $^{13}C/^{12}C$ ratio is less than that of the standard by 2.6 %. A comparison of the CO_2 δ values (control, Fig. 2) with the δ values characterizing each substrate (Table I) indicates that starch and/or protein is the respiratory substrate. A large body of ancillary information implicates starch[14].

When cyanide is added to the bathing medium the respiratory rate is not appreciably affected; however, the δ value of the respired CO_2 immediately begins to drop from the value typical of starch (Fig. 2). The change in the CO_2 δ value is sufficient to conclude that cyanide induces another respiratory carbon path. Furthermore, the increasing negative δ value with time is indicative of an ever-increasing participation of lipid degradation. It is noteworthy that when azide is given to avocado discs the respiratory quotient (R.Q.) drops markedly at the same time that the respiration is stimulated[18]. A drop in the CO_2 δ value and R.Q. is expected if cyanide

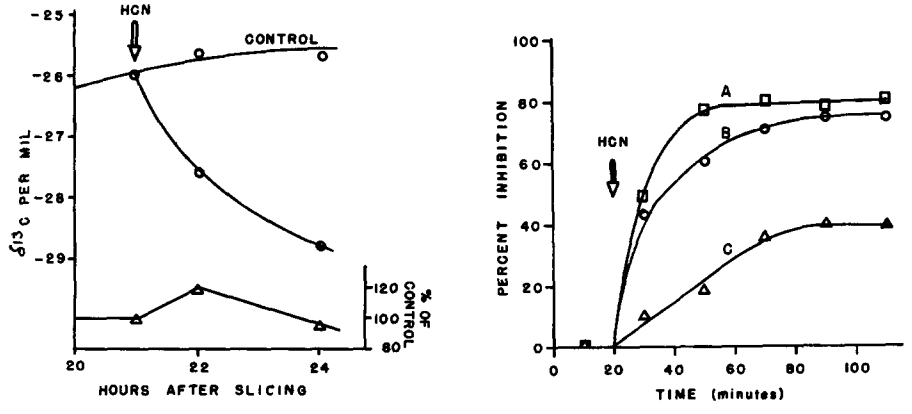

Fig. 2. Effect of HCN on the $\delta^{13}C$ of respired CO_2 from aged potato discs. Tissue was incubated in 10^{-4} M $CaSO_4$ (pH 5.0) for 20 h before use. HCN was added at an initial concentration of 0.22 mM. Lower curve (△—△), relative rate of respiration in the presence of cyanide. Upper curves (O—O), $\delta^{13}C$ per mil of respired CO_2.

Fig. 3. Cyanide inhibition of $^{14}CO_2$ evolution by aged potato discs provided with labeled substrates. Curve A, 5 μC [2,3-$^{14}C_2$]succinic acid; Curve B, 10 μC [$^{14}C_6$]glucose; Curve C, 10 μC [1-^{14}C]-myristic acid. Radioactive CO_2 was collected for 20-min intervals through 120 min. Cyanide, at an initial concentration of 0.3 mM, was added 20 min after the beginning of the experiment. Each point represents an average of two experiments.

causes a change from carbohydrate to lipid oxidation. From a knowledge of the δ values of the substrates and respiratory CO_2, it can be shown by conservation equations[14] that the relative contribution of lipid to the respired CO_2 is about 18 % in the absence of cyanide. After 2 h in cyanide, the lipid contribution is increased to 52 % of the total respired CO_2.

Nature of carbon pathway as determined by the oxidation of labeled substrates

While studies using the mass spectrometer lead to the conclusion that lipid is the substrate for compensatory respiration, a similar conclusion is at first glance not supported by experiments involving the metabolism of labeled substrates. For example, cyanide inhibits $^{14}CO_2$ evolution not only from [$^{14}C_6$]glucose, but to some extent from [1-^{14}C]myristate as well (Fig. 3). On the face of it one may deduce the oxidation of both substrates involves a common terminal oxidase, and by inference at least, part of a common carbon path. The respiration of aged potato slices is markedly malonate sensitive, and glucose metabolism in this tissue is almost certainly by way of the tricarboxylic acid cycle[19]. On the knowledge that the tricarboxylic acid cycle contributes in large measure to the normal respiration of aged slices, the oxidation of labeled succinate can be used as a means of testing the effectiveness of inhibitors of the tricarboxylic acid cycle. Insofar as cyanide inhibits succinate oxidation, it may tentatively be presumed that cyanide inhibits $^{14}CO_2$ evolution from glucose—and to some extent from myristate—by the inhibition of tricarboxylic acid cycle-associated oxidation (Fig. 2). By the same token it must be inferred that cyanide-resistant lipid degradation comprising compensatory respiration proceeds primarily by a path other than the tricarboxylic acid cycle. The evolution of $^{14}CO_2$ from ^{14}C-labeled fatty acids under conditions where the tricarboxylic acid cycle is blocked will for convenience be termed tricarboxylic acid cycle-independent oxidation.

If the large part of myristate oxidation which is unaffected by cyanide—*i.e.* tricarboxylic acid cycle-independent myristate oxidation—is to be taken as representative of cyanide-resistant lipid oxidation, it must be demonstrated that this pathway is stimulated when cyanide is given to potato tissue. To this end the percent of the total fatty acid degradation mediated on the one hand by the tricarboxylic acid cycle, and on the other by tricarboxylic acid cycle-independent oxidation, was determined for aged potato discs before and after the presentation of cyanide.

The $^{14}CO_2$ collected from tissue given [1-^{14}C]myristic acid is taken to arise both from tricarboxylic acid cycle-dependent and tricarboxylic acid cycle-independent oxidations, while the $^{14}CO_2$ evolved in the presence of a tricarboxylic acid cycle inhibitor represents the relative contribution of tricarboxylic acid cycle-independent metabolism. Malonate is used to block the tricarboxylic acid cycle in normal aged tissue. In the case of cyanide-induced compensatory respiration, cyanide itself serves as the inhibitor of the tricarboxylic acid cycle (Fig. 3). In both cases, corrections must be made for the effectiveness of the inhibitors in arresting the tricarboxylic acid cycle. These corrections are based on the percent inhibition by malonate and HCN, respectively, of the oxidation of [2,3-$^{14}C_2$]succinate. Insofar as a cyanide-resistant electron transport by-pass may in part implement tricarboxylic acid cycle-linked electron flow it is of paramount importance that the resistant path diverts from the normal one beyond the point where electrons from succinate oxidation join the common path[4]. Hence a correction for tricarboxylic acid cycle activity based on the

TABLE II

OXIDATION OF [1-^{14}C]MYRISTIC ACID AND [2,3-$^{14}C_2$]SUCCINATE BY AGED POTATO DISCS: EFFECTS OF MALONATE AND CYANIDE

Substrate	*Inhibitor*	$^{14}CO_2$ *(disint./min per 20 min)*	*Inhibition* (%)	*Uptake (disint./min* $\times 10^{-6}$)
Myristic acid	None	40 000	—	12.6
	50 mM malonate	13 500	66	—
	0.3 mM HCN	25 400	36	12.8
Succinic acid	None	13 500	—	0.85
	50 mM malonate	3 600	73	0.70
	0.3 mM HCN	2 600	81	0.90

influence of cyanide on succinate oxidation has the meaning imputed to it, in that both incomplete inhibition of cytochrome oxidase and electron diversion by way of a cyanide-resistant path are accounted for. Malonate was found to inhibit the oxidation of myristate by aged discs 66 % and of succinate, 73 % (Table II). Since the amounts of ^{14}C-labeled compounds taken up were not affected by malonate or cyanide, and far exceeded the quantity necessary to give the total $^{14}CO_2$ produced, the inhibition of $^{14}CO_2$ evolution is not due to inhibition of label uptake. When inhibition of myristate oxidation is corrected to take into account the incomplete effect of malonate on succinate oxidation, myristate oxidation is seen to be 90 % tricarboxylic acid cycle-mediated in normal tissue. When the extent of cyanide inhibition of myristate oxidation through the tricarboxylic acid cycle is similarly corrected by reference to the effect of cyanide on succinate oxidation, only 44 % of myristate oxidation appears to be tricarboxylic acid cycle-mediated in the presence of cyanide. Put another way, tricarboxylic acid cycle-independent metabolism of myristate is increased from 10 % in the absence of cyanide to 56 % in its presence (Table III).

TABLE III

RELATIVE CONTRIBUTION OF TRICARBOXYLIC ACID CYCLE-DEPENDENT AND -INDEPENDENT FATTY ACID OXIDATION BY NORMAL AND CYANIDE-TREATED AGED POTATO DISCS

Respiration	*Percent contribution*	
	Tricarboxylic acid cycle-dependent	*Tricarboxylic acid cycle-independent*
Normal	90	10
HCN-induced	44	56

Assuming that the relative contribution of the two types of myristate-oxidizing systems deduced from label experiments reflects the relative activities of these systems *in vivo*, the fraction that the various respiratory carbon paths contributes to the total respiration can be calculated. Such calculations suggest that the bulk of the CO_2 respired by aged potato discs comes from starch mediated primarily by glycolysis and the tricarboxylic acid cycle[19], with perhaps some involvement of the pentose

TABLE IV

RELATIVE CONTRIBUTION OF STARCH, TRICARBOXYLIC ACID CYCLE-DEPENDENT AND -INDEPENDENT LIPID DEGRADATION TO AGED DISC RESPIRATION AND CYANIDE-INDUCED RESPIRATION

Respiration	*Percent contribution*		
	Starch	*Lipid*	
		Tricarboxylic acid cycle-dependent	*Tricarboxylic acid cycle-independent*
Normal	82	16	2
HCN-induced	48	23	29

phosphate shunt[20]. The rest of the respired CO_2 comes from tricarboxylic acid cycle-dependent (16 %) and tricarboxylic acid cycle-independent (2 %) lipid metabolism. The salient feature respecting the effect of cyanide is that it fails to affect the respiratory rate while causing a tremendous increase in the contribution of tricarboxylic acid cycle-independent lipid oxidation (Table IV).

DISCUSSION

The values for the relative contributions of the various degradative pathways (Table IV) indicate that respiration in the presence of cyanide comprises a mixture of normal respiration mediated by the tricarboxylic acid cycle and tricarboxylic acid cycle-independent lipid oxidation. The persistence of an element of tricarboxylic acid cycle activity presumably is due to incomplete cyanide inhibition. Since the bulk of $^{14}CO_2$ evolved by potato discs given [2,3-$^{14}C_2$]succinate reflects oxidation of the latter by way of the tricarboxylic acid cycle, incomplete inhibition of $^{14}CO_2$ evolution from succinate by cyanide reflects incomplete inhibition of the cycle. Since succinate oxidation is only partially inhibited by 0.3 mM HCN (Fig. 3), it can be concluded the respiration in the presence of cyanide consists of a residuum of tricarboxylic acid cycle-mediated respiration together with cyanide-induced compensatory respiration.

While the effect of cyanide on potato discs is quite rapid as measured by the inhibition of glucose or succinate oxidation (Fig. 3), the cyanide-induced change of respiratory substrates, as indicated by a change in the CO_2 δ values, appears to be much slower (Fig. 2). If cyanide simultaneously inhibits tricarboxylic acid cycle-mediated carbohydrate oxidation, and stimulates tricarboxylic acid cycle-independent lipid breakdown, the decrease of CO_2 δ values should be as rapid as the inhibition of $^{14}CO_2$ evolution in label experiments (compare Figs. 2 and 3). As previously shown with 1 mm thick potato slices, it takes approximately 3 h to exchange 1 μmole of tissue HCO_3^- per g with respiratory CO_2, and it must be remembered that the δ value of tissue bicarbonate is greater than that of lipid engendered respiratory CO_2 by 14 per mil[14]. Therefore, the delayed change of CO_2 δ values upon cyanide addition is no doubt due to the slow exchange of previously dissolved CO_2 and HCO_3^- originating from starch, with the lighter CO_2 arising from lipid.

Though the exact nature by which lipids are oxidized during cyanide-induced

respiration is as yet unknown, a fatty acid-oxidizing system fulfilling the criteria established for the cyanide-induced system is the α-oxidation of long chain fatty acids. Malonate, which blocks the tricarboxylic acid cycle, does not inhibit the enzymes involved in the α-oxidation of long chain fatty acids[21]. Therefore, α-oxidation can function as a tricarboxylic acid cycle-independent fatty acid oxidative system. Furthermore, α-oxidation is resistant to moderate (1 mM) concentrations of cyanide[22], as is the tricarboxylic acid cycle-independent respiration. While tricarboxylic acid cycle-independent fatty acid oxidation appears to be stimulated by cyanide, it is doubtful the stimulation is direct. More likely stimulation is a consequence of cyanide blockage of one electron transport system with consequent evocation of another[4,5]. Thus, on two accounts α-oxidation might be the carbon path for the cyanide-induced compensatory respiration.

While the respiration of fresh discs is similar to that of cyanide-induced respiration in aged tissue in that both are probably mediated by a tricarboxylic acid cycle-independent fatty acid degradative system[14], the respiration of fresh discs is nevertheless inhibited by cyanide[3]. A possible explanation for cyanide inhibition on the one hand, and compensation on the other, is the comparative prevalence in fresh and aged tissue of a cyanide-resistant electron transport system. As aging progresses potato mitochondria are characterized by an increase in the ratio of cytochrome *b* to cytochrome a–a_3 (ref. 3). Furthermore, it is a *b*-type cytochrome that remains oxidized in the presence of cyanide[9,10]. All of the observations are consistent with the view that cyanide-resistant electron transport is due to a by-pass of the cyanide-sensitive cytochrome a_3 (*cf.* ref. 5). Most significant is the demonstration that aged discs have a relatively greater amount of the cyanide-insensitive component of the electron transport chain. Assuming that electron transport must be coupled with tricarboxylic acid cycle-independent fatty acid oxidation for continued CO_2 production and O_2 utilization, it is obvious that the inhibition of either component prevents the operation of the total system. Therefore, while cyanide-induced respiration of aged discs and the respiration of fresh discs probably involve the same carbon path, fresh disc respiration is sensitive to cyanide in the absence of a cyanide-resistant electron transport system.

In conclusion, it is evident that cyanide-resistant respiration is not a result of a mere indifference to the inhibitor, but is due to the suppression of one pathway and the evocation of another. A comparison of fresh tissue respiration with the cyanide-induced respiration of aged discs (see previous paragraph) suggests that for plant tissues to exhibit cyanide-induced compensatory respiration they must have a cyanide-resistant electron transport chain linked to a tricarboxylic acid cycle-independent lipid degradative system.

ACKNOWLEDGMENTS

This work was generously supported by a grant from the U.S. Public Health Service to G.G.L., and by the National Science Foundation Grant BG 7517 to S.E. B.S.J. is indebted to the U.S. Public Health Service for National Institute of Health Fellowship GM-33,256 from the National Institute of General Medical Sciences. This is contribution 1744 from the Division of Geological Sciences, California Institute of Technology.

REFERENCES

1 W. O. JAMES AND H. BEEVERS, *New Phytologist*, 49 (1950) 353.
2 I. R. MACDONALD, *Ann. Botany London*, 23 (1959) 241.
3 D. P. HACKETT, D. W. HAAS, S. K. GRIFFITHS AND D. J. NIEDERPRUEM, *Plant Physiol.*, 35 (1960) 8.
4 D. P. HACKETT, in B. WRIGHT, *Symp. Soc. Gen. Physiologists, 1961*, Ronald Press, New York, 1963, p. 105.
5 S. H. LIPS AND J. B. BIALE, *Plant Physiol.*, 41 (1966) 797.
6 G. G. LATIES, *Plant Physiol.*, 39 (1964) 391.
7 G. G. LATIES AND C. HOELLE, *Plant Physiol.*, 40 (1965) 757.
8 D. S. BENDALL, *Biochem. J.*, 70 (1958) 381.
9 D. S. BENDALL AND R. HILL, *New Phytologist*, 55 (1956) 206.
10 B. CHANCE AND D. P. HACKETT, *Plant Physiol.*, 34 (1959) 33.
11 C. LANCE AND W. D. BONNER, *Plant Physiol.*, 43 (1968) 756.
12 P. H. ABELSON AND T. C. HOERING, *Proc. Natl. Acad. Sci. U.S.*, 47 (1961) 623.
13 R. PARK AND S. EPSTEIN, *Plant Physiol.*, 36 (1961) 133.
14 B. S. JACOBSON, B. N. SMITH, G. G. LATIES AND S. EPSTEIN, *J. Gen. Physiol.*, 55 (1970) 1.
15 B. S. JACOBSON, *The Prevalence of Carbon-13 in Respiratory CO_2 as an Indicator of the Type of Endogenous Substrate in Potato Tissue*, Ph. D. Dissertation, Univ. Calif., Los Angeles, 1970.
16 R. PARK AND S. EPSTEIN, *Geochim. Cosmochim. Acta*, 21 (1961) 110.
17 H. CRAIG, *Geochim. Cosmochim. Acta*, 3 (1953) 53.
18 S. H. LIPS, *Electron Transfer Systems during Ripening of Avocado Fruits*, Ph. D. Dissertation, Univ. Calif., Los Angeles, 1964.
19 G. G. LATIES, *Plant Physiol.*, 39 (1964) 654.
20 T. AP REES AND H. BEEVERS, *Plant Physiol.*, 35 (1960) 839.
21 P. CASTELFRANCO AND P. K. STUMPF, *J. Biol. Chem.*, 214 (1955) 567.
22 P. K. STUMPF, *J. Biol. Chem.*, 223 (1956) 643.