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Membrane Lipid Breakdown in Relation to the Wound-induced and Cyanide-resistant Respiration in Tissue Slices

A COMPARATIVE STUDY[1]

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ABSTRACT

A study of a variety of bulky storage organs and fruits reveals that fresh slices fall into two categories with respect to their sensitivity to CN. Fresh slices in the first class are CN-sensitive, whereas slices of the second class are resistant to, and often stimulated by, CN. In tissue slices which are initially CN-sensitive, cutting initiates a burst of lipolytic activity. In CN-resistant fresh slices, there is no measurable lipid breakdown.

Slicing evokes the wound-respiration which is 5- to 10-fold that of the parent organ. Slice aging, in turn, evokes a further 2- to 3-fold respiratory increase, the wound-induced respiration, whether fresh slice respiration is CN-sensitive or -resistant. Estimation of the contribution by the cytochrome and alternative paths shows that the wound respiration in both groups is mediated by the cytochrome path. On the other hand, the wound-induced respiration in the first class is cytochrome path mediated, whereas, in some members of the second group, both pathways are utilized. Uncouplers of oxidative phosphorylation elicit a CN-sensitive increment in fresh slices as great or greater than the wound-induced respiration. Accordingly, *de novo* synthesis of mitochondria is ruled out as an explanation of the latter.

The integrity of endomembranes, perhaps including mitochondrial membranes, is seemingly a prerequisite for the operation of the alternative path, that is, alternative path activity is lost concomitantly with membrane lipid breakdown. The development of the wound-induced respiration is not coextensive with the development of the CN-resistant path in all tissue slices. The fundamental process of aging appears to involve activation of preexisting respiratory capacity.

Fresh slices from whatever source fail to utilize exogenous ^{14}C-labeled glucose, whereas aged slices do so readily. A transport lesion is indicated, the healing of which does not depend on the development of the wound-induced respiration but does depend on fatty acid, and presumably membrane lipid, biosynthesis.

Through the years, potato slices have become the prototypical bulky storage organ for slice physiology studies. Consequently, generalizations drawn from potato slice studies have usually served as a reference for studies of other tissue slices. However, the respiration of fresh potato slices has been shown to be anomalous (17). Their CN sensitivity has been attributed to the loss of the alternative path (27) due to extensive membrane lipid destruction initiated by slicing. Furthermore, regeneration of the CN-resistant pathway, and the burgeoning of glycolysis and the tricarboxylic acid cycle (12, 14) in aged potato slices were viewed as the result of specific membrane biosynthesis (33, 34). The question arises whether these physiological transitions are ubiquitous in tissue slices or unique to the potato slice.

Here, tissue slices from a variety of bulky storage organs and fruits were examined to determine whether slicing in general initiates a burst of lipolytic activity analogous to that found in potato and how slicing affects CN resistance and the utilization of exogenous glucose. The contribution of the alternative path of the wound and wound-induced respiration was estimated (2, 28, 29), as well as its magnitude, to establish the extent of its participation.

MATERIALS AND METHODS

PLANT MATERIAL

Potato tubers (*Solanum tuberosum* var. Russet) were grown by the Department of Vegetable Crops, University of California, Davis, and kindly provided by Professor Herman Timm. Potato tubers (*S. tuberosum* var. Desirée) were the gift of Professor T. Galliard of the Food Research Institute, Colney Lane, Norwich, England. Tubers were stored at 7 C and 90% RH. Avocado fruits (*Persea americana* Mill. var. Hass) were collected from a private orchard in Los Angeles. Untreated banana fruits (*Musa cavendishii* Lambert var. Valery) were shipped from Central America and obtained immediately upon off-loading.

The following storage organs were purchased from local markets and used immediately: sweet potato (*Ipomea batatas*), parsnip (*Pastinaca sativa*), carrot (*Daucus carota*), rutabaga (*Brassica napobrassica*), turnip (*Brassica rata*), radish (*Rhaphanus sativus*), Jerusalem artichoke (*Helianthus tuberosus*), red beet (*Beta vulgaris*), jicama (*Pachyrrhizus erosus*), horseradish (*Rorippa armoracia*), Daikon radish (*Rhaphanus sativus*), and ginger (*Zingiber*

Slices 1 mm thick were cut into ice water, rinsed with distilled H_2O and either used promptly as fresh slices or set to age on a rotary shaker in 0.1 mM $CaSO_4$ solution in Erlenmeyer flasks at 25 C (28).

RESPIRATORY MEASUREMENTS

Respiration was measured by conventional manometry at 25 C as previously described (28). Respiratory measurements in the presence of imidazole and CCCP[3] were carried out in 10 mM

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[3] Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; CLAM, *m*-chlorobenzhydroxamic acid.

phosphate buffer (pH 8.3) containing 0.1 mM $CaSO_4$ (17). $^{14}CO_2$ released from uniformly labeled glucose was measured as previously described (29).

ρ DETERMINATION

The capacity of the alternative path (V_{alt}) was estimated by measuring the respiration rate in the presence of 0.1 mM KCN, and the Cyt path capacity (V_{cyt}) was estimated by measuring the respiratory rate in the presence of 2 mM CLAM, the respiration resistant to CN and CLAM together, V_{res}, being subtracted in each case. The per cent of the maximal alternative path which operates in the absence of inhibitor was estimated and is designated ρ (2).

LIPID METHODOLOGY

Sample Preparation. For intact organs, one mature storage organ (*e.g.* potato, carrot, etc.) was used for each experiment to avoid tissue heterogeneity. The organ was washed thoroughly with distilled H_2O. Slices 1 mm × organ diameter (20 g fresh weight) were cut directly into boiling ethanol and boiled for 5 min. For slices, fresh slices 1 mm thick (20 g fresh weight) were prepared from the same organ and, 2 h after slicing, were boiled in ethanol for 5 min previous to lipid extraction.

Lipid Extraction. The ethanolic extraction extract was collected, and the tissue was homogenized in 3 volumes chloroform-methanol (2:1, v/v) in a VirTis blender for 10 min at room temperature. After filtration of the homogenate under vacuum, the residue was washed with 300 ml chloroform-methanol and rehomogenized in 2 volumes chloroform-methanol. The chloroform-methanol filtrates were combined with the ethanolic extract and were taken to dryness by rotary evaporation at 35 C. The crude lipid extract was washed according to Bligh *et al.* (6) and Folch *et al.* (9). The lipid-containing chloroform phase was evaporated to dryness and the residue was dissolved in benzene-ethanol (4:1, v/v) (11). Aliquots of the lipid solution were taken for total lipid phosphorus assay (4) and for total fatty acid analysis by GLC (22).

The methyl esters were separated on a prepacked stainless steel column (1.83 m × 0.64 cm) containing 15% HI-EFF-2BP ethylene glycol succinate on 80- to 100-mesh Chromosorb W (AW) (Applied Science Labs., Inc.). The absolute amount of each fatty acid relative to methyl palmitate was calculated from the area of the peak divided by the relative detector response for each component.

BIOCHEMICALS

Linoleic acid, linolenic acid, methyl palmitate, CCCP, and imidazole were obtained from Sigma. [U-^{14}C]Glucose was supplied by ICN. BCl_3-methanol (12% w/v) was purchased from Supelco. A standard mixture of fatty acid methyl esters was obtained from Applied Science Labs, Inc. CLAM was synthesized as previously described (31); 4-amino-3-hydroxyl-1-naphthalenesulfonic acid was purchased from J. T. Baker Chemical Co. All organic solvents used were of analytical grade.

RESULTS

LIPID CONTENT IN INTACT ORGANS AND FRESH SLICES

Table I compares the total lipid phosphorus and fatty acid content of intact organs with that of slices therefrom. Fresh slices fall into two categories. In the first group, wherein fresh slices are extensively CN-sensitive (Table III), slicing results in a significant loss of lipid-P and total fatty acids. In the second, CN-resistant group, there is no loss of lipid-P or fatty acids on slicing. The small drop in lipid-P in avocado and banana is not reflected in a drop in fatty acids and is considered to reflect experimental error. If the fatty acids in Table I derive solely from phospholipid, the molar fatty acid values should be twice the lipid phosphorus values. On this basis, they are in great excess. In potato, there is approximately 60% as much galactolipid as phospholipid, and both drop to the same fractional extent on slicing (data not shown). Potato tubers have negligible free fatty acid. When the potato phospholipid values in Table I are multiplied by 3.2 (× 1.6 to account for galactolipid, and × 2 to estimate the fatty acid content), the respective products are within 10% of the observed values. The same is true in turnip, rutabaga, and beet, albeit the relative prevalence of galactolipid has not been determined in these tissues. By contrast, in the members of the CN-resistant group, there is a marked excess of fatty acids over that expected from the phospholipid and galactolipid content (the latter estimated by the potato rubric). The implication is that tissues of the second group contain a significant amount of neutral lipid, *i.e.* triglycerides, an unexceptionable presumption in avocado (5).

The fatty acid composition of members of the two groups is set out in Table II. The feature of note is the high level of linolenic acid in the CN-sensitive group. The value in beets, although somewhat low, often runs higher and invariably exceeds the level of 18:3 in banana.

CN RESISTANCE IN FRESH SLICES

Table III describes the CN sensitivity of fresh slices from various storage organs. Fresh discs can be classified into two categories. The first group comprises slices sensitive to CN, whereas slices of the second group are either significantly resistant to CN or even stimulated by it. Plant tissue slices have a respiratory component, the residual respiration (V_{res}), that is resistant to CN and hydroxamate (*e.g.* CLAM) together. When V_{res} (Table IV) is subtracted from the CN-resistant values in Table III, the distinction between the two groups is further magnified. In the CN-sensitive group, the CLAM-sensitive alternative path is essentially absent in fresh slices, whereas, in the CN-resistant group, fresh slices are characterized by a readily demonstrable alternative path (Table IV). It is for this reason that different tissues that occasionally display a similar gross resistance to CN are placed in different categories (*e.g.* red beet [*cf.* ref. 13] and carrot phloem [Table III]). It is the hydroxamate sensitivity of the CN-resistant respiration that dictates assignment to the CN-resistant category. A comparison of Tables I and II suggests that, when slicing causes a loss of lipid-P, fresh slice respiration is sensitive to CN, whereas, when fresh slices are CN-resistant, there is little or no membrane lipid destruction.

CN RESISTANCE IN TISSUE SLICES AS A FUNCTION OF AGING

Table III indicates the course of CN resistance with aging in initially CN-sensitive and CN-resistant slices. In the former case, CN resistance increases sharply with time, whereas, in the latter case, CN resistance actually drops. Once again, if V_{res} is subtracted, the changes in the CN-resistant alternative path are even more pronounced. As noted in Table III, wound-induced respiration occurs in all slices whether initially CN-sensitive or -resistant. When the CN resistance of aged slices is total, as in potato, the reconstitution of the alternative path concomitantly with the development of the induced respiration creates the illusion that the latter is sustained by the CN-resistant path. This misconception has been applied to storage organ slices in general. As will be seen, the increase in respiratory activity represents the fundamental change associated with slice aging. Whether the respiration is seen to be CN-sensitive or -resistant depends on the method of measurement.

CONTRIBUTION OF ALTERNATIVE PATH IN FRESH AND AGED SLICES

CN-sensitive Groups. Table IV shows the quantitative relations of V_T, V_{cyt}, V_{alt}, and V_{res}, as well as the value of ρ (28, 30) in fresh

Table I. *Total Lipid Phosphorus and Fatty Acid Content in Intact Organs and Fresh Slices*

Fresh slices indicate slices 2 h after cutting. Tissue group headings designate CN sensitivity of fresh slices.

Tissue	Lipid Phosphorus		Loss	Fatty Acids		Loss
	Intact	Fresh Slices		Intact	Fresh Slices	
	μmol/kg fresh wt		%	*μmol/kg fresh wt*		%
CN-sensitive						
Potato						
var. Russet	618	500	19	2,200	1,813	18
var. Desirée	895	788	12	3,239	2,684	17
Turnip	720	511	29	2,149	1,325	38
Rutabaga	940	639	32	3,243	2,121	35
Red beet	721	551	24	2,420	1,765	27
CN-resistant						
Sweet potato	1,400	1,435	0	5,526	5,982	0
Parsnip	1,857	1,815	2	11,364	11,649	0
Carrot	1,543	1,564	0	6,569	7,110	0
Avocado	3,055	2,890	5	400,690	400,390	0
Banana	883	829	6	6,972	7,029	0

Table II. *Fatty Acid Composition of Total Lipids from Various Intact Plant Organs*

Tissue group headings designate CN sensitivity of fresh slices. Avocado and banana fruit are preclimacteric.

Tissue	Fatty Acid				
	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
			%		
CN-sensitive					
Potato					
var. Russet	21	4	4	47	24
var. Desirée	22	4	1	51	22
Turnip	21	2	11	19	47
Rutabaga	20	1	8	11	59
Horseradish	15	1	9	30	44
Red beet	22	0.5	9	55	14
CN-resistant					
Sweet potato	35	7	Trace	50	7
Parsnip	18	Trace	10	66	5
Carrot	22	Trace	2	68	8
Avocado[a]	14	0.5	60	17	2
Banana[a]	39	3	11	31	11

[a] Avocado and banana also have 5% 16:1.

and aged slices originally either CN-sensitive or CN-resistant. In the first group, fresh slices lack the alternative path, as indicated by $V_{alt} = 0$. The bulk of fresh slice respiration in this group is mediated by the Cyt path. Fresh slice respiration in the absence of respiratory inhibitors (V_T) is the sum of V_{cyt} and V_{res}. The alternative path appears with aging, as indicated by a value of V_{alt} greater than zero. The wound-induced respiration in all cases but one in the first group proceeds through the Cyt path. The contribution of the alternative path is nil, as indicated by $\rho = 0$. In aged red beet slices, the one exception, the alternative path appears to be fully engaged with $\rho = 1$.

CN-resistant Group. The presence of the alternative path in fresh slices of the second group is demonstrated by values of V_{alt} greater than zero (Table IV). The contribution of the alternative path to fresh slice respiration is zero, however, since $\rho = 0$. Thus, fresh slice respiration is once again mediated exclusively by the Cyt path.

Although wound-induced respiration characterizes all slices (compare V_T values of fresh and aged slices), V_{alt} increases with aging in initially CN-sensitive tissue, whereas, in initially CN-resistant tissue, V_{alt} either drops or changes little. In spite of significant V_{alt} values, aged slices from initially CN-sensitive tissue do not use the alternative path in the absence of CN. By contrast, most aged slices from initially CN-resistant tissues do (see ρ values in Table IV).

Effect of Uncouplers on Fresh Slice Respiration. Table V shows the effect of CCCP on the respiration of fresh slices from the CN-sensitive and -resistant groups. Aging increases fresh slice respiration 2- to 5-fold to yield the wound-induced respiration. A comparison of coupled aged slice respiration with uncoupled fresh slice respiration reveals that, except for potato, the wound-induced respiration utilizes but 50 to 70% of the respiratory capacity of fresh tissue slices. Together with the observation that slicing *per se* evokes a respiration rate 3 to 10 times that of the parent organ, this comparison suggests a re-examination of the view that the wound-induced respiration depends upon *de novo* synthesis of additional respiratory units, *viz.* the mitochondria (1). It seems that aging activates and utilizes pre-existing respiratory capacity without the biogenesis of new mitochondria (28, 29).

Imidazole Inhibition of Uncoupler-stimulated Respiration of Fresh Tissue Slices. Since uncoupler stimulates the respiration of both groups of tissue slices (Table V), stimulation is seemingly independent of lipid degradation in fresh tissue slices. Nevertheless, the evidence suggests (16) that the uncoupled respiration of fresh potato slices, where lipid destruction is extensive, is not tricarboxylic acid cycle-related but, rather, represents α-oxidation of long-chain fatty acids. This conclusion has been based primarily on the observation that imidazole, a specific inhibitor of fatty acid α-oxidase (17), inhibits the uncoupled respiration in fresh potato slices. To determine whether the above conclusion, drawn from potato slices, holds in general for tissue slices that suffer extensive lipid destruction, the effect of imidazole was tested on the uncoupled respiration of a variety of fresh CN-sensitive slices. Where lipid degradation is not evident, *viz.* in CN-resistant fresh slices, imidazole is expected to be without effect.

Table VI affirms that imidazole severely inhibits the uncoupled respiration in tissue slices where lipid destruction is extensive. By contrast, imidazole is without effect on slices which are initially CN-resistant, show no lipid degradation, and presumably display no fatty acid α-oxidase activity.

Exogenous Glucose Utilization by Fresh and Aged Tissue Slices. The manifestation of fatty acid α-oxidase activity, as well as the inability of fresh potato slices to oxidize exogenous glycolytic and tricarboxylic acid cycle intermediates, has been attributed to the inhibition of glycolysis and the tricarboxylic acid cycle by free

Table III. *Development of Induced Respiration in CN-sensitive and CN-resistant Slices*

Tissue group headings designate CN sensitivity of fresh slices. Banana and avocado fruits are preclimacteric.

Tissue	Fresh			Aged			ΔCN/Δ Control
	Control	CN	Resistance	Control	CN	Resistance	
	μl O_2/g fresh wt·h		%	μl O_2/g fresh wt·h		%	%
CN-sensitive							
Potato							
var. Russet	35	10	28	135	135	100	125
var. Desirée	53	14	26	176	180	102	134
Radish	33	1	3	70	33	47	86
Daikon radish	59	7	12	66	30	45	328
Turnip	74	3	4	113	78	69	192
Rutabaga	75	10	13	117	62	53	124
Red beet	58	17	29	138	40	29	28
Jerusalem artichoke	44	18	41	105	50	48	52
Jicama	16	5	31	51	54	105	140
CN-resistant							
Sweet potato							
Red	82	149	181	138	134	97	−26
Yellow	110	90	82	198	127	64	42
Parsnip							
Phloem	105	112	106	170	46	27	−101
Xylem	67	97	145	150	54	36	−52
Carrot							
Phloem	117	34	29	142	16	11	−72
Xylem	79	34	43	124	34	27	0
Banana	63	66	104	102	62	61	−10
Avocado	145	178	123				
Ginger	35	38	108				

Table IV. *Respiratory Parameters of Fresh and Aged Slices*

Tissue group headings designate CN sensitivity of fresh slices.

Tissue	Fresh						Aged					
	V_T	V_{alt}	V_{cyt}	V_{res}	V_{alt}/V_T	ρ	V_T	V_{alt}	V_{cyt}	V_{res}	V_{alt}/V_T	ρ
	μl O_2/g fresh wt·h						μl O_2/g fresh wt·h					
CN-sensitive												
Potato												
var. Russet	38	0	28	10	0	0	144	92	105	39	0.64	0
var. Desirée	47	0	39	8	0	0	155	127	100	45	0.82	0
Radish	58	0	52	5	0	0	89	17	85	3	0.18	0
Daikon radish	74	0	71	5	0	0	130	12	110	18	0.09	0
Turnip	44	0	38	10	0	0	90	10	78	12	0.11	0
Rutabaga	81	11	65	15	0.1	0	162	48	120	40	0.30	0
Red beet	99	0	80	12	0	0	141	16	117	10	0.11	1
Jerusalem artichoke	41	5	25	14	0.1	0	105	30	90	21	0.29	0
Jicama	16	0	10	4	0	0	55	49	51	5	0.89	0
CN-resistant												
Sweet potato	68	85	46	26	1.25	0	115	75	89	30	0.65	0
Parsnip												
Phloem	106	130	95	10	1.22	0	219	30	165	20	0.14	1
Xylem	88	30	66	20	0.34	0	162	32	125	8	0.20	1
Carrot												
Phloem	100	106	96	11	1.06	0	131	17	100	18	0.13	0.8
Xylem	68	10	60	12	0.15	0	145	36	117	18	0.25	0.3
Banana	60	53	45	13	0.88	0	105	45	97	17	0.43	0

fatty acids released during cutting (27). Aging has been shown to increase exogenous glucose utilization some 3,000-fold (14), presumably due to the deinhibition of glycolysis and the tricarboxylic acid cycle attending the oxidative scavenging of the aforementioned free fatty acids (17). Accordingly, it might be anticipated that CN-resistant fresh slices, free of demonstrable lipid breakdown, would prove able to oxidize exogenous glucose.

Table VII compares the evolution of radioactive $^{14}CO_2$ from [U-^{14}C] glucose by fresh and aged slices from initially CN-sensitive and CN-resistant tissues, respectively. Contrary to expectations,

Table V. *Effect of Uncouplers on Respiration of Fresh Slices from Various Tissues*

Tissue group headings designate CN sensitivity of fresh slices. Avocado and banana fruit are preclimacteric.

Tissue	Intact Organ	Fresh Slices	Fresh +CCCP	Stimulation by CCCP	Aged Slices	Aged/Uncoupled Fresh
	μl CO_2/g fresh wt·h			%	*μl O_2/g fresh wt·h*	%
CN-sensitive						
Potato						
var. Russet	8	35	128	365	135	105
var. Desirée	14	53	119	226	176	145
Rutabaga	10	75	203	270	117	58
Turnip	12	74	190	256	113	59
Red beet	14	58	199	343	138	69
CN-resistant						
Red sweet potato	20	82	195	237	138	70
Parsnip phloem	21	105	281	267	170	60
Carrot phloem	12	112	245	218	142	58
Avocado	40	115	235	204	147	62
Banana	10	63	184	292	102	55

Table VI. *Response to Imidazole of Coupled and Uncoupled CN-sensitive and CN-resistant Fresh Slices*

Tissue group headings designate CN sensitivity of fresh slices. Avocado and banana fruit are preclimacteric.

Tissue	Control	KCN, 0.1 mM	Imidazole, 0.1 M	CCCP, 10 μM	CCCP + Imidazole
	μl O_2/g fresh wt·h				
CN-sensitive					
Potato					
var. Russet	47	10	36	128	42
var. Desirée	44	9	32	119	37
Turnip	99	12	60	190	112
CN-resistant					
Aged potato (Russet)	143	172	100	220	180
Red sweet potato	117	154	130	251	233
Parsnip phloem	150	136	162	281	270
Carrot phloem	112	81	109	245	252
Avocado	148	150	120	235	209
Banana	111	83	123	184	142

Table VII. *Absorption and Oxidation of Radioactive Glucose by Initially CN-sensitive and CN-resistant Slices*

Initial radioactivity of external solution was 18.2×10^6 [U-^{14}C]glucose dpm/15 ml. Experimental period was 2 h. Group headings designate CN sensitivity of fresh slices. Avocado fruit is preclimacteric.

Tissue	$^{14}CO_2$ Release			Uptake	
	Fresh	Aged	Relative Rate (Aged/Fresh)	Fresh	Aged
	dpm × 10^{-4}/3 g fresh wt·h			*% initial radioactivity*	
CN-sensitive					
Potato	0.24	120	500	6	94
Turnip	1.8	128	71	15	93
Red beet	19.1	150	8	38	95
CN-resistant					
Red sweet potato	0.43	108	251	16	95
Parsnip	0.44	91	207	12	86
Avocado	0.71	47	66	2	97
Carrot	0.56	60	107	10	83

all fresh tissue slices fail to release significant amounts of $^{14}CO_2$ from exogenous glucose. Aging enhances exogenous glucose utilization 70- to 500-fold. Glucose absorption is significant even in fresh tissue. Since uptake is so extensive in aged slices, however, a comparison of absorption rates based solely on an initial and final measurement of the experimental solution has little meaning.

At first glance, it seems that the development of the capacity for exogenous substrate utilization by aging slices is inextricably linked to the development of the wound-induced respiration as heretofore suggested (14). To our surprise, we have observed that maximal rates of exogenous substrate utilization develop in slices aged in actinomycin or cycloheximide (data not shown), agents that totally inhibit the development of the wound-induced respiration (7). An interpretation of this unexpected phenomenon is offered under "Discussion."

DISCUSSION

CN RESISTANCE IN RELATION TO MEMBRANE INTEGRITY

Whereas the respiration of the parent organs of both groups of slices in this study is CN-resistant or even stimulated by CN (26), CN resistance is lost immediately on cutting in one group, whereas, in the other group, CN resistance survives slicing. Where CN resistance persists in fresh slices, there is no measurable lipid breakdown, whereas, where CN resistance is lost, lipid destruction is demonstrable. Since it is phospho- and galactolipid that disappears, there is every reason to assume that it is membrane lipid that is being degraded. Considering that the location of the alternative path is in the mitochondria (8, 31), it may be deduced that some alteration in one or more mitochondrial membrane lipids leads to the inactivation of the alternative path (10, 27, 32).

The absence of lipid breakdown in CN-resistant fresh slices might be due to the absence of lipolytic enzymes, to the presence of an acyl-hydrolase inhibitor, or to the unsusceptibility, for whatever reason, of membrane lipoprotein to lipid acyl-hydrolase action. An effective acyl-hydrolase inhibitor has been discovered in carrot tissue, where there is no lipid destruction in crude homogenates or in tissue slices (ref. 30; *cf.* Table I). Carrot acyl-hydrolase inhibitor is a heat-labile protein of mol wt greater than 10,000 that inhibits isolated potato and yeast lipid acyl-hydrolases as well as lipolysis in potato homogenates (*cf.* refs. 24 and 25). The physiological effects of lipid degradation in CN-sensitive fresh slices are reasonably to be attributed to the breakdown of

membrane phospho- and galactolipids. The presence of significant levels of triglycerides in CN-resistant tissues raises the possibility of a protective or sparing action of the latter on phospho- and galactolipid destruction.

SLICE AGING AND CN RESISTANCE

It has been axiomatic that the development of the alternative path is co-extensive with that of the wound-induced respiration (12, 15, 18, 23). The study presented here shows that the above relationship is not invariable. CN resistance develops with aging only in initially sensitive slices. In initially resistant slices, the induced increment is CN-sensitive, V_{alt} either remaining the same or dropping. Whether fresh slices are CN-sensitive or -resistant, respiration is enhanced with slice aging. The induced respiration in all cases represents a basic enhancement of respiratory activity. If the lesion in the alternative path is remedied during aging, the CN-resistant respiration, when quantitatively equal to the induced respiration as in potato, seemingly comprises the induced increment. In fact, in the absence of CN, respiration proceeds through the Cyt path. The CN sensitivity perceived in aged slices initially resistant to CN is attributed to a decrease of V_{alt} and a simultaneous increase in respiratory flux with aging. Since V_{cyt} is greater than V_{alt} in such aged slices, CN inhibition is observed. In all tissues, fresh slice respiration is mediated exclusively via the Cyt path since $\rho = 0$. In CN-sensitive slices, this is to be expected since V_{alt} is zero. However, ρ remains zero in CN-resistant fresh slices despite the fact that V_{alt} often exceeds V_{cyt}. In aged slices of the CN-sensitive group, ρ frequently remains zero, whereas ρ often is 1 or nearly 1 in aged slices of the initially CN-resistant group. In such cases, the Cyt and alternative paths operate simultaneously. The engagement of the alternative path reflects the inability of the Cyt path to accommodate the total enhanced flux due to aging. Maximal V_{cyt} values (measured in the presence of uncoupler) in fresh and aged potato slices are approximately the same (28, 29). Accordingly, the elevated observable Cyt-mediated rates of aged slices are not to be attributed to the *de novo* synthesis of Cyt components and, hence, of mitochondria. Rather, aging involves the expression of existing respiratory capacity, *i.e.* aging entails substrate mobilization. When substrate mobilization exceeds the capacity of the Cyt path in aged slices, the engagement of the alternative path is observed. In some tissues, this occurs only in the presence of uncoupler (28, 29). Altogether, the data support Bahr and Bonner (2, 3) in the view that the level of traffic in the Cyt chain controls the operation of the alternative path.

Are CN-resistant Fresh Slices Physiologically Similar to Aged, Induced, CN-resistant Slices? Because of the compelling correlation between measurable lipid breakdown and the loss of CN resistance which attends cutting in many bulky storage organs, it might be expected that fresh CN-resistant slices would resemble aged CN-resistant slices derived from initially sensitive fresh slices. Such is not the case. As pointed out, all fresh slices, from whatever source, fail to utilize exogenous substrates. When slices of any sort are aged in cerulenin, an inhibitor of fatty acid synthesis (34), the normally developed ability to utilize exogenous substrate is absent, as is the induced respiration. The invariable dependence on aging in this connection, together with the cerulenin sensitivity of the process, suggest effective localized lipid degradation in CN-resistant fresh slices, albeit below the level of reliable measurement.

In this view, the cycloheximide- and actinomycin-indifferent scavenging of free fatty acids with time relieves carbon path inhibition. Appropriately, potato slice aging in imidazole prevents the development of the induced respiration (S. D. Grover, unpublished). As scavenging proceeds, over-all cerulenin-sensitive phospholipid synthesis regenerates critical membrane components, which may bear upon the integrity of glucose and organic acid transporters. The result is perceived in the ability of slices to use exogenous substrate with or without the wound-induced respiration. The respiration of sweet potato slices held in cerulenin for 24 h stays steady, implying no dearth of substrate. There is not a sufficient drop in lipid content to sustain the noted respiration rate through 1 day, and it must be deduced that carbohydrate is the primary substrate. Thus, the persisting inability to utilize exogenous substrate implicates transport impairment, a proposition reinforced by the observed ability of banana slices to oxidize exogenous acetate at the same time that they fail to oxidize exogenous glucose or citrate (20). The augmentation of metabolic flux, on the other hand, the very basis of the wound-induced respiration, depends on RNA and protein synthesis—as does repair of the lesion in the alternative path—and accordingly is inhibited by actinomycin cycloheximide (7).

Effect of Uncouplers on Fresh Tissue Slices. Low levels of free fatty acids have been shown to inhibit the mitochondrial ADP/ATP translocator, thus limiting electron transport much as would oligomycin (35). The stimulatory effect of CCCP on fresh potato slice respiration has been attributed by us to a release of such an oligomycin-like inhibition of oxidative phosphorylation by free fatty acids. A similar explanation may apply to tissues such as turnip and rutabaga, for example, where free fatty acids are released by cutting. The basis of uncoupler stimulation of fresh CN-resistant slices is more problematical, since measurable lipid breakdown is absent in these tissues. Nevertheless, as suggested above, lipid breakdown, although diminished, may be localized and thereby highly effective, with the ensuing free fatty acid levels high enough to affect carbon path metabolism and phosphorylation but not high enough to disrupt the alternative path.

The respiration of intact bulky storage organs is normally suppressed. First, fresh slice respiration is 3 to 10 times that of the intact organ, and is elevated another 2 to 4 times by uncouplers. Second, cyanide stimulates intact organ respiration manyfold (26). Thus the alternative path as well as the Cyt path is under profound restraint in the intact organ.

Why then is the respiration of intact organs so low? The capacity for very much higher rates is there. O_2 tension appears not to be at issue, since the oxygen tension in the potato tuber is well above the $K_m^{O_2}$ for Cyt oxidase, and higher external O_2 tensions fail to raise the respiration (19). Endogenous volatiles (21) have yet to be ruled out as a regulatory parameter, despite the absence of convincing evidence of their involvement. The trigger for the wound and wound-induced respiration in thin slices of bulky organs remains to be elucidated.

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