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THE α-OXIDATION OF LONG-CHAIN FATTY ACIDS AS A POSSIBLE COMPONENT OF THE BASAL RESPIRATION OF POTATO SLICES

G. G. LATIES and CAROL HOELLE

Division of Plant Physiology, Department of Botanical Sciences, University of California, Los Angeles

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Abstract—Fresh potato slices evolve $^{14}CO_2$ from carboxyl-labeled palmitic and myristic acids. Shorter chain fatty acids are not perceptibly metabolized except for propionate. By contrast, aged potato slices vigorously oxidize fatty acids from C_{16} to C_2. The oxidation of palmitate and myristate by fresh tissue is malonate resistant, while fatty acid oxidation by aged tissue is largely malonate sensitive. The suggestion is made that α-oxidation is responsible for the overt degradation of the appropriate long-chain fatty acids in fresh slices, while β-oxidation gains ascendancy with aging. The extensive labeling of citrate by carboxyl-labeled fatty acids in fresh slices points to the formation of acetyl-CoA. However, since the tricarboxylic acid cycle is inoperative in fresh tissue, acetyl-CoA is not metabolized, and β-oxidation of fatty acids is consequently not observed. In fresh tissue propionate is metabolized predominantly by a modified β-oxidation wherein propionate is first decarboxylated, ultimately to yield acetyl-CoA wherein the carboxyl carbon is C-3 of propionate. With aging the oxidation of propionate proceeds increasingly by CO_2 fixation via methyl malonyl-CoA and succinyl-CoA. The evidence suggests both pathways of propionate breakdown operate simultaneously, the latter path becoming more prevalent with time.

INTRODUCTION

PLANT tissues frequently exhibit more than one respiratory pathway. The endogenous respiration of a given tissue may be enhanced in a variety of ways, the resulting respiratory increment frequently exceeding the initial respiration. The respiration of fibrous roots is sharply increased by dilute salt solutions;[1] *Chlorella* cells may respond to glucose;[2] uncouplers of oxidative phosphorylation stimulate respiration in a variety of tissues.[1] In each case there is reason to suspect that the respiratory increment is different in kind from the initial respiration, and the concept has arisen of a basal respiration in plants, upon which may be superimposed an induced or developed respiration.[3,4] When thin slices of fleshy storage organs are aged, the respiration gradually rises through some 24 hr until the respiratory rate is three to four times that of the fresh slice.[3] The respiration which develops with time is clearly different from the initial respiration, and the distinguishable respiratory characteristics of fresh and aged storage organ slices perhaps best exemplify the concept of a distinct basal and developed respiration.[4]

The respiration which develops with time in potato tuber slices comprises characterizable carbon paths including the Embden–Meyerhof glycolytic pathway, the tricarboxylic acid cycle,[5] and the pentose phosphate pathway.[6,7] To date *nothing* has been revealed regarding

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[2] R. EMERSON, *J. Gen. Physiol.* 10, 469 (1927).
[3] G. G. LATIES, *Plant Physiol.* 37, 679 (1962).
[4] G. G. LATIES, In *Control Mechanism in Respiration and Fermentation*, p. 129. Ronald Press (1963).
[5] G. G. LATIES, *Plant Physiol.* 39, 654 (1964).
[6] T. APREES and H. BEEVERS, *Plant Physiol.* 35, 839 (1960).
[7] J. A. ROMBERGER and G. NORTON, *Plant Physiol.* 36, 20 (1961).

the carbon path of basal respiration in this tissue, and it appears likely that the same can be said for many other tissues as well. As measured by the evolution of $^{14}CO_2$ from carbon-labeled substrates, fresh potato slices oxidize neither glucose, fructose, hexose phosphates nor sucrose—nor do they utilize acetaldehyde, ethyl alcohol, amino acids, glycolate, glyoxylate, glycerol or any di- or tricarboxylic acids of the tricarboxylic acid cycle.[5] Fresh slices are malonate resistant. The evidence points to an inability to oxidize isocitrate,[5] with the result that tricarboxylic acid cycle activity is curtailed with attendant and presumably consequential inhibition of glycolysis.[8]

The respiratory quotient of fresh potato slices is roughly 0·7, and approaches 1·0 with time. Preliminary experiments (R. Park and G. G. Laties, unpublished) have shown that the $^{13}C/^{12}C$ ratio of the endogenous respiratory CO_2 drops markedly in the first hours after slicing and subsequently increases, signifying changes in respiratory substrate. The $^{13}C/^{12}C$ ratio of potato lipid is considerably lower than that of total tissue carbon. Studies with aged potato slices indicate that when the normal respiration path is blocked by an inhibitor a compensatory respiration may be elicited which draws on a different endogenous substrate.[9] Furthermore, inhibitors of electron transport may markedly lower the R.Q. in certain plant tissues without suppressing oxygen utilization.[10] The foregoing observations taken together suggested that fat or fatty acid oxidation may be involved in basal respiration, and perhaps in induced respiration under certain circumstances as well. Since, however, basal respiration in potato slices is malonate resistant, and since, furthermore, fresh slices cannot oxidize the ultimate product of β-oxidation of fatty acids (e.g. acetate), the possibility was examined that fresh slices carry out the α-oxidation of long-chain fatty acids.[11] Although the concentration of lipids in potato tubers is only about 0·25 per cent of the dry weight,[12] the respiration of intact tubers is also very low.

RESULTS AND DISCUSSION

Fatty Acid Oxidation by Fresh Tissue

Fresh potato slices metabolize certain of the long-chain fatty acids in a way which implicates α-oxidation. Thus while myristate is readily oxidized, and palmitate noticeably so, neither laurate nor any shorter-chain fatty acids release $^{14}CO_2$ from the carboxyl position (Table 1). The specificity is much the same as that described by Castelfranco *et al.*[13] (cf. Refs. 14 and 15). β-Oxidation of carboxyl-labeled fatty acids will yield carboxyl-labeled acetyl-CoA, as will the oxidation of pyruvate-2-^{14}C. In Table 2 a comparison is made of myristate-1-^{14}C and pyruvate-2-^{14}C oxidation by fresh and aged potato slices. While fresh slices utilize myristate they fail to oxidize pyruvate. Furthermore, myristate oxidation is markedly malonate sensitive in aged slices, but resistant in fresh. The bulk of the enhancement of myristate utilization brought about by aging is malonate sensitive, malonate dropping the rate of $^{14}CO_2$ evolution in aged disks essentially to that found in fresh. Pyruvate oxidation by aged disks is virtually totally malonate sensitive (note the effect of malonate on acetate oxidation,

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[13] P. CASTELFRANCO, P. K. STUMPF and R. CONTOPOULOU, *J. Biol. Chem.* **214**, 567 (1955).
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Table 3). The data suggest that α-oxidation is solely responsible for overt myristate degradation in fresh slices, while in aged slices β-oxidation, followed by TCAC-mediated oxidation of the acetate so formed, is superimposed on the basal level of α-oxidation.

TABLE 1. OXIDATION OF CARBOXYL-LABELED FATTY ACIDS BY FRESH POTATO SLICES

Acid	$^{14}CO_2$ Evolution		Radioactivity external solution	
	Control	Malonate	Initial	Final
	dpm × 10^{-2}/15 min		dpm × 10^{-6}	
Palmitic	17	26	7·0	5·0
Myristic	79	64	8·0	4·0
Lauric	*c.* 0	*c.* 0	10·8	1·0
Decanoic	*c.* 0	*c.* 0	8·4	0·5

3·0 g fresh weight in 15 ml solution. Controls, 0·05 M K phthalate, pH 5·0. Malonate treated, 0·05 M malonate, pH 5·0 (no phthalate). Rate of $^{14}CO_2$ evolution represents average for the first three 15-min periods. Rates corrected for blanks, i.e. label evolution in the absence of tissue. Evolution from octanoic, heptanoic, butyric, acetic equal or less than comparable blank. Blanks as follows (dpm/15 min): palmitic, 350; myristic, 450; lauric, 10,000; decanoic, 10,000 (also: octanoic, 15,000; heptanoic, 7000; butyric, 5000; propionic, 4000; pyruvic, 1500; acetic, 6000. See text).

TABLE 2. COMPARISON OF PYRUVIC-2-^{14}C AND MYRISTIC-1-^{14}C OXIDATION BY FRESH AND AGED POTATO SLICES

	$^{14}CO_2$ Evolution					
	Pyruvic-2-^{14}C		Myristic-1-^{14}C			
			Fresh		Aged	
Time	Fresh Control	Aged Control	Control	Malonate	Control	Malonate
Min			dpm × 10^{-2}/20 min			
20	0	118	50	46	236	94
40	0	400	86	79	522	136
60	0	610	71	72	521	91
Acid	Radioactivity of organic acids					
	dpm × 10^{-2}					
Citric	15	6	113	5	6	13
Malic	65	562	91	14	340	35
PCA*	42	262	9	33	167	—
Succinic	86	54	76	200	53	160

* Pyrrolidone-2-carboxylic acid.

TABLE 3. OXIDATION OF CARBOXYL-LABELED FATTY ACIDS BY AGED POTATO SLICES

	$^{14}CO_2$ Evolution															
	Palmitic		Myristic		Lauric		Decanoic		Octanoic		Heptanoic		Butyric		Acetic	
Time	C*	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M
Min	dpm × 10^{-2}/15 min															
15	109	36	178	108	62	23	286	52	560	0	173	0	1650	35	650	38
30	183	37	226	107	84	—	769	100	1450	0	350	4	4720	93	1780	89
45	186	39	202	79	450	117	1060	165	2400	30	420	16	6850	32	2710	140
60	129	36	188	69	582	149	1230	193	2900	90	440	31	7400	47	3400	144
	Radioactivity external solution															
	dpm × 10^{-6}/15 ml															
Initial	7·4		7·4		10·6		8·9		25·0		3·2		19·8		18·8	
Final	4·8	4·2	3·5	3·7	0·9	0·7	0·8	0·9	3·5	5·7	1·3	1·5	3·2	7·2	6·3	9·2
	Initial concentration external solution															
	M × 10^5															
	1·6		8·0		7·8		8·0		6·0		3·0		5·2		1·2	

* C represents control; M, malonate treated.

Fatty Acid Oxidation by Aged Tissue

Aged potato slices readily oxidize fatty acids of chain lengths from C_{16} to C_2 (Table 3). Evolution of $^{14}CO_2$ from the shorter chain acids is almost totally inhibited by malonate, while a perceptible malonate resistance is noted with palmitate, myristate and perhaps with laurate. The foregoing observation supports the hypothesis that long-chain (C_{16}–C_{14}) fatty acid utilization in aged tissue comprises α- and β-oxidation, while the oxidation of shorter chain fatty acids is solely of the β type.

The influence of malonate on the labeling of TCAC organic acid by aged disks (Table 4) further bolsters this assumption, malonate causing an accumulation of label in succinate, and a diminution of label in malate and pyrrolidone carboxylic acid (see Ref. 5). The extensive labeling of citrate in fresh tissue is noteworthy (Table 4). In aged tissue citrate is labeled to a considerably lesser extent than malate or pyrrolidone carboxylic acid, while in fresh tissue citrate is labeled in very great excess over malate and pyrrolidone carboxylic acid. The change in labeling pattern with aging is not attributable to changes in the quantity of endogenous acids.[5] The absence of tricarboxylic acid cycle activity in fresh potato slices has previously been attributed to an inability of the latter to oxidize citrate.[5] The extensive labeling of citrate in fresh tissue in response to carboxyl-labeled fatty acid, on the one hand reflects the noted impairment of citrate oxidation, and on the other suggests that β-oxidation *per se*, i.e. acetyl-CoA formation, does in fact occur in fresh tissue. The extent to which it occurs cannot be estimated from the data. Thus it is the absence of tricarboxylic acid activity which obscures and probably limits β-oxidation in fresh material.

TABLE 4. THE LABELING OF ORGANIC ACIDS BY CARBOXYL-LABELED FATTY ACIDS IN FRESH AND AGED POTATO SLICES

	Palmitic				Myristic				Lauric				Decanoic			
	Fresh		Aged		Fresh		Aged		Fresh		Aged		Fresh		Aged	
	C*	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M
	dpm × 10^{-2}															
Citric	272	174	50	39	460	90	200	40	1300	420	450	110	2780	460	880	150
Malic	45	153	180	44	47	9	—	23	130	20	550	63	262	29	1500	126
PCA†	21	153	120	39	23	17	—	35	38	34	346	90	363	245	1320	400
Succinic	25	192	32	220	30	106	12	103	98	348	208	344	156	630	210	340

	Octanoic				Heptanoic				Butyric				Acetic			
Citric	4050	764	4240	445	550	96	200	41	29,000	4840	4640	730	20,500	2470	2830	590
Malic	305	68	4630	310	68	18	403	26	2160	245	8300	780	1160	215	4290	454
PCA	150	104	3050	400	20	9	207	46	540	415	8500	1090	412	170	3220	386
Succinic	234	835	1010	1170	60	180	597	235	1000	4580	1500	2820	580	1430	720	1045

* C represents control; M, malonate treated.
† Pyrrolidone-2-carboxylic acid.

Although labeling of tricarboxylic acid cycle acids is more extensive in aged than in fresh tissue, labeling is nevertheless considerable in fresh slices and influenced by malonate, particularly with respect to label accumulation in succinate. All of which points up the admonition that when pools are relatively small and turnover times relatively rapid in comparison with the duration of the experimental period, labeling of metabolic intermediates may prove a poor quantitative measure of the contribution of the path in question to the total metabolism.[9] Nevertheless some labeling differences do exist. The extensive labeling of citrate in fresh tissue has been pointed out. The relatively small influence of malonate on pyrrolidone carboxylic acid labeling in fresh slices may also be noted.

Propionate Metabolism.

Propionate metabolism warrants special attention since propionate oxidation in plants has been shown to proceed by a unique 'modified β-oxidation' mechanism[16, 17] in which propionyl-CoA is oxidized first to β-hydroxy propionate, then to malonyl semialdehyde—following which further oxidation leads to malonyl-CoA and finally to acetyl-CoA. In this scheme the carboxyl group of propionate (C-1) is lost first in the events which lead to the formation of acetyl-CoA. C-3 of propionate becomes the carboxyl group of the acetyl residue, and C-2 the methyl group. Thus the sequence of appearance of the carbon atoms of propionate in respiratory CO_2 is C-1, C-3, C-2. Table 5 points to the occurrence of modified β-oxidation of propionate in potato slices. First, C-1 is evolved much more readily than is C-3 in fresh slices, and furthermore C-1 release is essentially malonate resistant, while C-3 release is markedly malonate sensitive. With aging, $^{14}CO_2$ evolution from all three carbons of propionate is enhanced. However, the evolution of $^{14}CO_2$ from C-3 remains more malonate sensitive than that from C-1. If propionate metabolism proceeded by modified β-oxidation exclusively, there would be no reason

[16] J. GIOVANELLI and P. K. STUMPF, *J. Biol. Chem.* 231, 411 (1958).
[17] M. D. HATCH and P. K. STUMPF, *Arch. Biochem. Biophys.* 96, 193 (1962).

to expect malonate sensitivity of propionate-C-1 oxidation in any event. Nevertheless, aged disks *do* show such sensitivity. The answer to this potential anomaly is to be found in the inordinately heavy labeling of succinate shown in Tables 5 and 6. The latter points to propionate carboxylation—presumably to methyl malonyl-CoA, and thence to succinyl-CoA—as in mammalian systems (see Ref. 18).

TABLE 5. THE OXIDATION OF SPECIFICALLY LABELED PROPIONATE BY FRESH AND AGED POTATO SLICES

	Propionate-1-^{14}C				Propionate-3-^{14}C			
	Fresh		Aged		Fresh		Aged	
	Control	Malonate	Control	Malonate	Control	Malonate	Control	Malonate
Min	$^{14}CO_2$ Evolution (dpm × 10^{-3}/15 min)							
15	16	19	44	8	*c.* 0	*c.* 0	9	2
30	60	54	91	15	*c.* 0	*c.* 0	23	2
45	66	50	119	23	*c.* 0	*c.* 0	45	2
60	102	75	144	25	*c.* 0	*c.* 0	44	3
Acid	Organic acid label (dpm × 10^{-3})							
Citrate	12	14	24	6	101	39	48	18
Malate	32	16	53	8	53	15	175	22
PCA	123	78	427	47	106	88	503	143
Succinate	207	255	231	257	201	230	88	57
	External solution (dpm × 10^{-6})							
Initial	11·8	10·9	14·3	14·5	15·8	15·6	18·8	16·5
Final	5·4	5·9	7·4	8·8	7·9	8·3	9·8	11·0

Initial external concentration: $2{\cdot}5 \times 10^{-4}$ M; 2·7 mc/mmol.

Although methyl malonyl-CoA has been observed in plant tissues as a product of propionate carboxylation,[19] there has been no evidence to date of methyl malonyl-CoA conversion to succinate, and the vitamin B_{12} dependence of the isomerization in bacteria and in mammalian tissue has further cast doubt on the likelihood of this transformation in higher plants. Nevertheless the expectations attending succinate formation via methyl malonyl-CoA from specifically labeled propionate are met in potato slices, and the possibility must be left open that succinate can be formed in this way in potato. If the labeling of the dicarboxylic acids by propionate were solely by way of acetyl-CoA, propionate-1-^{14}C would not be expected to label the 4-carbon dicarboxylic acids to any considerable degree.[17] However, in fresh slices TCAC acid labeling by propionate-1-^{14}C is essentially as great as by propionate-3-^{14}C (Table 5), and in aged slices acid labeling by propionate-1-^{14}C remains considerable, albeit less than by propionate-2 or propionate-3-^{14}C. It is further to be expected that pro-

[18] P. R. VAGELOS, *Ann. Rev. Biochem.* 33, 139 (1964).
[19] M. D. HATCH and P. K. STUMPF, *Plant Physiol.* 37, 121 (1962).

pionate-2-^{14}C and propionate-3-^{14}C will be equally effective in labeling the TCAC acids if labeling occurs via methyl malonyl-CoA and succinate. Table 6 verifies that such is the case, and, together with Table 5, establishes not only that labeling of the dicarboxylic acids is extensive from propionate-1-^{14}C as well, but that more label is incorporated into the TCAC acids from propionate-1-^{14}C than is evolved as $^{14}CO_2$ (cf. Ref. 17). The extensive labeling of succinate in particular by propionate-1-^{14}C suggests that the carboxylation and isomerization reactions occur more rapidly than does modified β-oxidation. The labeling of the TCAC acids in general while $^{14}CO_2$ evolution is still limited (propionate-2-^{14}C, Table 6) again points to the likelihood of a small active pool of cycle intermediates (see above).

TABLE 6. THE OXIDATION OF SPECIFICALLY LABELED PROPIONATE BY AGED POTATO SLICES

	Propionate-1-^{14}C		Propionate-2-^{14}C		Propionate-3-^{14}C	
	Control	Malonate	Control	Malonate	Control	Malonate
Min	$^{14}CO_2$ Evolution (dpm × 10^{-3}/15 min)					
15	14	7	*c.* 0	*c.* 0	*c.* 0	*c.* 0
30	34	16	*c.* 0	*c.* 0	13	*c.* 0
45	48	21	4	*c.* 0	23	1
60	66	23	7	*c.* 0	39	3
	Organic acid label (dpm × 10^{-3})					
Citrate	9	5	26	12	28	15
Malate	14	6	116	16	96	23
PCA	63	55	296	58	225	84
Succinate	231	177	794	105	344	335
	External solution (dpm × 10^{-6})					
Initial	13·1	14·4	16·2	15·0	20·7	20·8
Final	6·2	8·4	5·3	8·1	6·2	10·0

Initial external concentration: Propionate-1-^{14}C, $2{\cdot}5 \times 10^{-4}$ M, 2·7 mc/mmol; propionate-2-^{14}C, $1{\cdot}3 \times 10^{-4}$ M, 5·2 mc/mmol; propionate-3-^{14}C, $1{\cdot}4 \times 10^{-4}$ M, 4·7 mc/mmol.

Potato slices do not oxidize malonate as evidenced by the total absence of $^{14}CO_2$ evolution from malonate-2-^{14}C.[5] Furthermore, malonate totally inhibits $^{14}CO_2$ production from propionate-2 or propionate-3-^{14}C, while inhibiting $^{14}CO_2$ release from propionate-1-^{14}C hardly at all in fresh tissue, and but to a limited extent in aged. The evidence suggests, therefore, that malonate is acting as an inhibitor of succinic dehydrogenase, and hence of the TCAC, and is not exerting its major influence by pre-empting the available endogenous CoA in reactions involving the formation of malonyl-CoA.[19] Nevertheless it is not excluded that to some extent malonate impairs succinate labeling by propionate by competing for available CoA. The failure of labeled succinate to accumulate in response to malonate when propionate provides the label (Tables 5, 6), in contrast with the marked accumulation of

labeled succinate in response to malonate when carboxyl-labeled fatty acids provide the label (Table 4), may reflect this additional effect of malonate. More to the point, however, is the observation that malonate inhibits the conversion of methyl malonyl-CoA to succinate.[20]

In aged tissue, propionate may be oxidized through the tricarboxylic acid cycle presumably either via acetyl-CoA, following modified β-oxidation, or by way of succinate, following carboxylation and isomerization. In the presence of malonate, release of C-1 is less in aged than in fresh slices, even though the rate of $^{14}CO_2$ evolution of the controls has risen sharply (Table 5). Thus there would appear to be a pre-emptive diversion of propionate to succinate in tissue in which the TCAC is operative, i.e. in aged tissue. The operation of the tricarboxylic acid cycle in aged slices allows an examination of the relative rates of release of C-1, C-2 and C-3, from propionate. The relative rates of release are in the order C-1, C-3, C-2 (Table 6) as expected on the basis of modified β-oxidation. The malonate sensitivity of C-1 release nevertheless indicates some propionate oxidation via the carboxylation pathway, suggesting that aged tissue oxidizes propionate in both ways.

EXPERIMENTAL

Potato disks 1·0 mm thick and 9·0 mm in diameter were cut from Russet Burbank tubers as previously described.[3] Aged disks were incubated in 10^{-4} M $CaSO_4$ for 24 hr at 25° on a rotary shaker. Long-chain carboxyl-labeled fatty acids (New England Nuclear) were brought into solution as described by Castelfranco *et al.*[13] Control experimental solutions were 0·05 M with respect to phthalate, pH 5·0 in all cases. Malonate-containing solutions, free of phthalate, were 0·05 M malonate, pH 5·0. 15 ml solution containing 10 μc ^{14}C-carboxyl-labeled fatty acid was held in 125 ml Erlenmeyer flasks. 3·0 g fresh weight (45 disks) was placed in each flask, and shaken in a reciprocal shaker at 25°. Malonate-treated tissue was exposed to malonate for 30-40 min before being placed in malonate-containing isotope solutions.

A strip of Whatman glass-paper (GF/A) 1 cm × 8 cm was suspended as a loop from a hook in a No. 4 rubber stopper. The glass-paper was impregnated with 0·2 ml 10 per cent KOH, and hung 2–3 in. above the level of solution when the rubber stopper was fixed in position. At prescribed intervals the stoppers and paper were removed from the flask and a standby stopper and impregnated paper substituted immediately. Radioactive alkali papers were dried in an oven at 80° and thereafter dropped directly into scintillator fluor for estimation of radioactivity.

There is some volatilization of fatty acid in the absence of potato tissue leading to blanks of some 300 dpm/15 min for myristate to a maximum of 15,000 dpm/15 min for octanoate. Blanks reflected the combined influences of volatility and water solubility. Blank corrections were made in one of two ways. Experimental solutions were shaken with alkali papers for several intervals before addition of tissue, or else experimental flasks without tissue were run concomitantly. Blank values were found to be essentially proportional to the solution concentration. Since uptake of fatty acids from solution is rapid, blank values for flasks containing tissue change during the experimental period. Thus, blanks from empty flasks frequently proved higher than the total rate of label evolution (respiratory plus blank) from flasks containing tissue. In such cases evolution of $^{14}CO_2$ by the tissue was presumed to be very low—and given as "*c.* 0" in the tables. On occasion the initial and final radioactivity of the external solution was noted, and, on the assumption of a roughly linear decrease with time, an estimation of the blank at each interval was made as a function of the concentration of the

[20] M. FLAVIN and S. OCHOA, *J. Biol. Chem.* **229**, 965 (1957).

external solution. In almost all cases, $^{14}CO_2$ evolution from fatty acid respiration was so far in excess of the blank, with active tissue, and the escape of radioactive volatiles so close to the blank with inactive tissue, that no ambiguity arose. The bulk of the blank presumably comprised the acids themselves, since neither previous flushing with CO_2 nor evaporation and resuspension of the fatty acid stock solutions subsequently lessened the blank. Aliquots (0·1 ml) of the external solution were applied to alkali-impregnated glass-papers and dried and counted in the same way as radioactive CO_2.