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Inhibition of the Development of Induced Respiration and Cyanide-insensitive Respiration in Potato Tuber Slices by Cerulenin and Dimethylaminoethanol[1]

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ABSTRACT

The interdependence of the development of wound-induced respiration and membrane-related phospholipid biosynthesis in potato tuber (*Solanum tuberosum* var. Russet) slices was established by the use of agents which selectively affect lipid and phospholipid synthesis. Cerulenin, a specific inhibitor of *de novo* fatty acid synthesis, inhibited the ultimate development of wound-induced respiration and of cyanide resistance only when given in the critical first 10 to 12 hours of slice aging. Similarly, when slices were exposed to the choline analogue dimethylaminoethanol within the first 10 hours, the phospholipid composition of the membrane lipids was drastically altered, the wound-induced respiration in a 24-hr period was substantially curtailed, and the development of cyanide insensitivity was sharply inhibited. These observations indicate that time-restricted membrane-related phospholipid synthesis is prerequisite to the development of wound-induced respiration and concurrent cyanide insensitivity.

Fresh slices of a variety of bulky storage organs undergo a time-dependent respiratory rise—the wound-induced, or induced, respiration. The development of induced respiration through a 24-hr period is suppressed by actinomycin, an inhibitor of DNA-directed RNA synthesis, in a time-restricted manner. That is, actinomycin is inhibitory only when given in the first 8 to 10 hr of the 24-hr period following cutting. In the preceding paper (21), it was demonstrated that the time-restricted characteristic of actinomycin action applied also to the effect of actinomycin on choline incorporation into phospholipid, and selectively to the proliferation of three enzymes of lecithin biosynthesis, *viz.* phosphorylcholine-cytidyl transferase, phosphorylcholine-glyceride transferase, and phosphatidylphosphatase. The view was developed that membrane-related phospholipid synthesis is central to the development of induced respiration, and that the respiratory changes are a consequence of membrane biosynthesis and membrane intussusception into existing mitochondria.

In this paper, we examine the effect of two specific and sensitive inhibitors of normal lipid biosynthesis within the framework of the time-restricted concept (see ref. 21). The intent is to distinguish between events which are prerequisite to wound-induced respiration and those which are merely coincident with it. The rationale is that metabolic elements which are inhibited in a time-restricted way—as is respiration by actinomycin—are primary events in the genesis and course of induced respiration. We have investigated the effect of cerulenin and DME,[2] respectively, on the onset and course of the developed respiration as a function of the time of inhibitor presentation in the 24 hr following cutting. Cerulenin (2S) (3R) 2,3 expoxy-4-oxo-7,10 dodecadienoylamide, an antibiotic produced by the fungus *Cephalosporium coerulens*, specifically inhibits fatty acid synthetase (1, 3). Dimethylaminoethanol serves as an analogue of choline and in that role is incorporated into an analogue of lecithin (7, 20). In what follows, it is shown that both of these agents inhibit the development of wound-induced respiration in a time-restricted manner, and it is deduced accordingly that phospholipid, and by extension membrane, biosynthesis are central elements in respiratory development in aging slices.

MATERIALS AND METHODS

Preparation and Treatment of Plant Tissue. Potato tubers were kindly supplied by H. Timm, Dept. of Vegetable Crops, University of California, Davis. Potato tuber slices (*Solanum tuberosum* var. Russet) were prepared as previously described, and aged in calcium sulfate solution (21).

The antibiotic cerulenin was the generous gift of S. Ōmura, of the Kitasato Institute, School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan. Stock solutions (1 mg/ml) were prepared in a minimal volume of absolute ethanol (1 mg cerulenin/30 μl ethanol). Aliquots were diluted with 0.1 mM calcium sulfate solution to obtain the desired concentration.

Stock solutions of 0.1 M dimethylaminoethanol (Aldrich Chemical Co.) were brought to a pH of 8 with 5 N HCl prior to addition to the incubation medium. The final medium was 1 to 10 mM depending on the storage history of the tubers.

Oxygen uptake of slices was measured by manometry or with the O_2 electrode.

Application of Radioactive Precursors to Tissue Disks. The incorporation of [1-^{14}C]acetate (50 mCi/mmol, Cal Atomic) into fatty acids and sterols was accomplished by incubating 10 disks in a ml of 0.1 mM $CaSO_4$ solution with 2 μCi of labeled material. The method used to determine the incorporation of [1,2-^{14}C]choline (21 mCi/mmol, New England Nuclear) into phospholipid in aging potato disks was essentially the same as that employed for [^{14}C]acetate. Incorporation of L-[U-^{14}C]leucine (270 mCi/mmol, New England Nuclear) into protein in potato tissue was assayed by the method of Laties (12).

Extraction, Isolation, and Separation of Lipids. The total tissue lipids were extracted and isolated by the isopropyl alcohol-chloroform-methanol procedure described previously (21).

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[2] Abbreviations: DME: dimethylaminoethanol; PDME: phosphotidyldimethylethanolamine.

Separation and Characterization of Individual Fatty Acids. Further fractionation of the lipid extract of both radioactive and nonradioactive samples was accomplished as follows after flash evaporation of the chloroform phase to a minimal volume of approximately 3 ml. The concentrated lipid sample was transferred to a screw cap culture tube and evaporated under N_2. Two ml of 1 N NaOH in methanol was added to the residue, the tube flushed with N_2, sealed with a Teflon-lined cap, and the sample saponified in a boiling water bath for 2 hr. The solution was then cooled, adjusted to 50% methanol by adding 2 ml of water, and the nonsaponified fraction extracted from the aqueous phase with three 4-ml aliquots of petroleum ether, leaving the fatty acids in the methanolic phase.

Fatty acid analysis was accomplished by methylation of the fatty acids in the aqueous methanol solution. The fatty acids were removed from the methanol phase by addition of 4 ml of 1.5 N HCl followed by petroleum ether extraction. The petroleum ether extract was taken to dryness under N_2. The fatty acids were esterified with BCl_3-methanol according to Metcalfe *et al.* (13). The methyl esters were then separated with an F & M model 819-19 gas chromatograph equipped with a 2.4 m stainless steel column having 10% diethylene glycol succinate on Chromosorb W 60/80 mesh, and operated with a dual flame detector. The injection port was kept at 230 C while the column was operated isothermally at 185 C, and the detector at 230 C.

Isolation and Characterization of Phospholipids. Phospholipid analysis of the tissue proceeded with the evaporation of the total lipid extract to a minimal volume and the separation of the concentrated lipids by TLC. Glass plates were spread with a 500-μm layer of Silica Gel H (Applied Science Labs.) which was activated at 110 C for 1 hr before use. The total phospholipids were separated from sterols, galactolipids, and free fatty acids by use of the solvent system of Gardner (6) which consisted of acetone-water-acetic acid (100:1:0.5, v/v). The phospholipids which remained at the origin were scraped from the plate, eluted from the silica gel with 20 volumes of chloroform-methanol-concentrated ammonium hydroxide (36:13:3, v/v), and concentrated under N_2. The phospholipid concentrate was then spotted on a Silica Gel H plate and the individual phospholipids were resolved with either chloroform-methanol-water (65:25:4, v/v) or chloroform-methanol-ammonium hydroxide (36:13:3, v/v) according to Waechter and Lester (20). Phospholipids were identified by including standard phosphatidylcholine (Applied Science Labs.) and phosphatidylethanolamine (Sigma Chemical Co.) as references. The various phospholipids (samples as well as standards) were located by exposing the plates to iodine vapor. Lipid phosphorous was estimated by digestion of the isolated phospholipid with 72% perchloric acid followed by colorimetric determination of phosphorous (5).

The fatty acid content of the individual phospholipids was determined by first masking part of the plate with Saran Wrap and exposing the plate to iodine vapor in order to locate the specific phospholipids. The Saran Wrap was then removed, and the unexposed areas scraped off using the iodine-treated areas as a reference. The phospholipids were then eluted from the gel with 20 volumes of chloroform-methanol-ammonium hydroxide (36:13:3, v/v), brought to dryness under N_2, and then saponified and methylated by the same technique utilized for the determination of total fatty acids.

The incorporation of radioactive precursors into the various lipid fractions was measured with a liquid scintillation counter. Samples were suspended in scintillation fluid according to the method of Kates (10).

Table I. The Effect of Cerulenin on the Respiratory Rate and Cyanide Sensitivity of Potato Disks Aged for 24 Hr

Sample	Respiratory Rate
	$\mu l\ O^2\ g^{-1}\ hr^{-1}$
Fresh slice	19
Control, 24-hr aged	110
Control, 24-hr aged + 0.22 mM HCN	129
Cerulenin, 24-hr aged	28
Cerulenin, 24-hr aged + 0.22 mM HCN	19

Cerulenin: 100 μg/ml

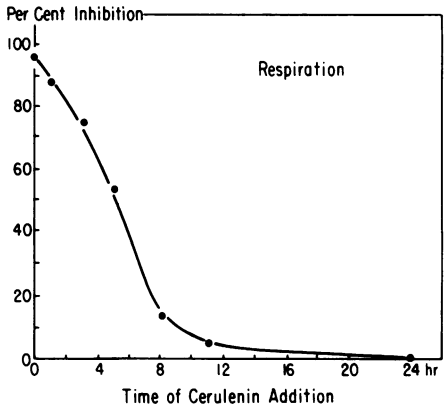

FIG. 1. Effect of time of addition of cerulenin (100 μg/ml) on the developed respiration rate of potato disks. Inhibitor was added after 0, 1, 2.5, 5, 8, or 11 hr of incubation. Measurement of the respiratory rate was determined at the end of 24 hr.

RESULTS

Effect of Cerulenin on the Development of Induced Respiration. The possibility that *de novo* fatty acid synthesis was prerequisite to the induced respiration was tested by treatment of potato tuber slices with a specific inhibitor of fatty acid synthesis, cerulenin.

When potato disks were treated with 100 μg/ml cerulenin from the outset, the respiration rate after 24 hr was similar to that of the freshly sliced material (Table I). Cerulenin-aged disks also showed cyanide sensitivity, a condition normally associated only with freshly sliced tissue, while control aged disks displayed characteristic cyanide insensitivity.

If cerulenin was added at various times after slicing, the resulting respiratory pattern based on the measurement at 24 hr shows a progressive decrease in the inhibitory effect of cerulenin additions within the first 11 hr (Fig. 1). After 11 hr of aging, addition of cerulenin had no inhibitory effect on the development of wound-induced respiration.

Effect of Cerulenin on Fatty Acid Metabolism. Confirmation of cerulenin's specific effect on fatty acid synthesis was determined by the effect of cerulenin on radioactive acetate incorporation into lipid, as well as by analysis of the total amount and composition of fatty acid in the treated tissue. The incorporation of [1-^{14}C]acetate into the total tissue lipid at 8 hr was drastically inhibited by cerulenin (Table II). It is in the first 8 to 10 hr that fatty acid synthesis exerts its greatest effect on the development of induced respiration (Fig. 1). When the total lipid extract was separated into saponifiable and nonsaponifiable fractions (Table II), the fatty acid fraction was found to have by far the greatest amount of label incorporation when compared with the nonsaponifiable extract. The saponifiable fraction showed the same amount of inhibition of label incorporation in the presence of cerulenin as that found in the total lipid extract.

The degree of inhibition of incorporation of labeled acetate

Table II. The Effect of Cerulenin on the Incorporation of [1-^{14}C]Acetate into Cell Lipid Fractions

Radioactivity in the total lipid fraction, the free fatty acid and nonsaponifiable fatty acid of 8-hr old potato disks pulsed simultaneously with cerulenin (100 μg/ml) and [1-^{14}C]acetate (2 μCi/ml) for 2 hr.

	Label Incorporation		Inhibition
	Control	Cerulenin	
	cpm		%
Total lipid	55,080	6,926	87.4
Free fatty acid	44,334	6,055	86.3
Nonsaponifiable fatty acid	2,890	1,466	49.2

Table III. The Effect of Cerulenin on [1-^{14}C]Acetate Incorporation into Total Lipid during the Course of Aging

The effect of addition of cerulenin (100 μg/ml) and [1-^{14}C]acetate (2 μCi/ml) for a 2-hr pulse during different intervals of the aging process.

Time	Label Incorporation in Total Lipid		Inhibition
	Control	Cerulenin	
hr	cpm		%
0 - 2	10,531	1,569	85.9
10 - 12	37,994	3,405	91.0
22 - 24	42,216	7,148	83.1

Table IV. The Effect of Cerulenin on the Total Amount of Fatty Acid and Fatty Acid Composition of Fresh, Aged, and Cerulenin Aged Potato Disks

Potato disks were incubated for 24 hr in cerulenin (100 μg/ml).

Sample	Total Fatty Acid	14:0	16:0	16:1	18:0	18:1	18:2	18:3
	μg fatty acid/g fresh wt.							
Fresh	179.6	4.0	34.8	4.0	13.2	5.2	91.2	27.2
Aged	224.6	2.0	40.0	4.2	17.2	9.6	106.8	44.8
Cerulenin Aged	114.6	2.4	28.0	2.4	9.6	3.6	46.6	22.0

into fatty acid was observed to be similar during all intervals of aging in the first 24 hr (Table III).

Analysis of the total fatty acid (unlabeled) content of the tissue revealed differences between fresh, aged, and cerulenin-aged material (Table IV), a reassuring affirmation that the effect of cerulenin is on fatty acid synthesis *per se* and not on the uptake of labeled precursor (see Table II). Tissue extracted immediately after slicing had less total fatty acid than that extracted after 24 hr of aging in calcium sulfate solution (*cf.* ref. 18). Cerulenin-aged samples showed a decline in fatty acid in comparison with both fresh and aged samples. Aging influenced the total fatty acid composition of the potato tissue. In particular, there appeared to be increased amounts of 18:1, 18:2, and 18:3 fatty acids with aging, whereas cerulenin-aged slices resembled fresh slices in terms of the fatty acid composition.

Effect of Cerulenin on the Incorporation and Uptake of Choline into Phosphatidylcholine. With cerulenin present throughout the 24 hr of aging, choline incorporation during a 2-hr pulse at 24 hr was limited to that observed in fresh slices. Later times of exposure of the tissue to cerulenin resulted in the characteristic pattern of time-restricted inhibition with regard to curtailment of choline incorporation into phospholipid (Fig. 2). The sensitivity of choline uptake to cerulenin reflected the effect of cerulenin on choline incorporation.

Effect of Cerulenin on the Incorporation of [U-^{14}C]Leucine into Protein. Another index of the specific inhibition of fatty acid synthesis by cerulenin can be shown by comparing the effect of cerulenin on [U-^{14}C]leucine incorporation into protein, and [1-^{14}C]acetate incorporation into lipid, during the 8- to 10-hr interval of the critical period.

The possible effects of cerulenin on protein synthesis were investigated by giving cerulenin and labeled L-leucine to disks in a 2-hr pulse 8 hr after cutting (Table V). During the same time interval, other tissue samples were pulsed with labeled acetate in the presence or absence of cerulenin. It can be seen in Table V that the effect of cerulenin on protein synthesis in the period in question was significantly less than the inhibitory action of cerulenin on *de novo* fatty acid synthesis, as demonstrated by the limited [1-^{14}C]acetate incorporation into lipid.

Effect of Dimethylaminoethanol on the Development of Induced Respiration. The augmented incorporation of labeled choline into phosphatidylcholine (17) which coincides with the manifestation of induced respiration suggests that the respiratory increase in aging tissue disks is linked with specific phospholipid formation. The possibility that specific phospholipid polar head groups are a requirement for the development of the augmented respiration with age was examined by exposure of tissue slices to choline analogues in the incubation medium during the time course of aging. In particular, DME was used as an analogue of choline in order to inhibit the incorporation of the latter into phosphatidylcholine.

The development of respiration in tissue disks treated at the outset with DME (1-10 mM depending on the storage history of the tubers) and exposed to the latter for the 24-hr period of aging was significantly inhibited (Table VI). Disks aged in DME also showed extreme sensitivity to cyanide, in contrast to the behavior of aged control tissue (Table VI).

Addition of DME at progressively later times resulted in a decrease in its inhibitory effect on the attainment of the induced

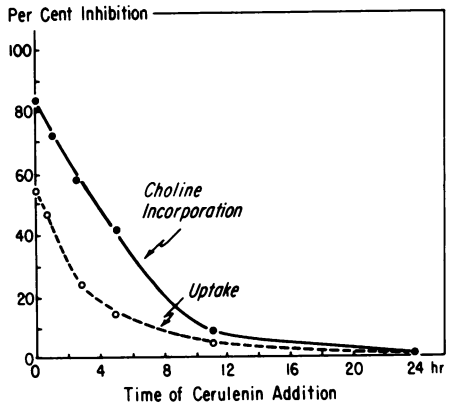

FIG. 2. Effect of delayed addition of cerulenin (100 μg/ml) on the uptake and incorporation of [1,2-^{14}C]choline (0.5 μCi/ml) into phosphatidylcholine. Inhibitor was added after 0, 1, 2.5, 5, or 11 hr of incubation. Measurement of the rate of uptake and incorporation was made in a 2-hr interval at the end of 24 hr.

Table V. The Effect of Cerulenin on Protein and Fatty Acid Synthesis

The effect of cerulenin (100 μg/ml) on [U-^{14}C]leucine incorporation into protein compared with [1-^{14}C]acetate incorporation into total lipid during a 2-hr pulse in the interval 8 to 10 hr after slicing. L-leucine: 0.5 μCi/ml; [1-^{14}C]acetate: 2 μCi/ml.

	Label Incorporation		Inhibition
	Control	Cerulenin	
	cpm		%
Protein	24,373	16,020	34.3
Total lipid	27,540	3,460	87.4

Table VI. The Effect of Dimethylaminoethanol on the Respiratory Rate and Cyanide Sensitivity of Aged Potato Disks

Sample	Respiratory Rate
	ul O_2 g^{-1} hr^{-1}
Fresh slice	23
Control, 24-hr aged	121
Control, 24-hr aged + 0.1 mM HCN	112
DME (10 mM), 24-hr aged	77
DME (10 mM), 24-hr aged + 0.1 mM HCN	45

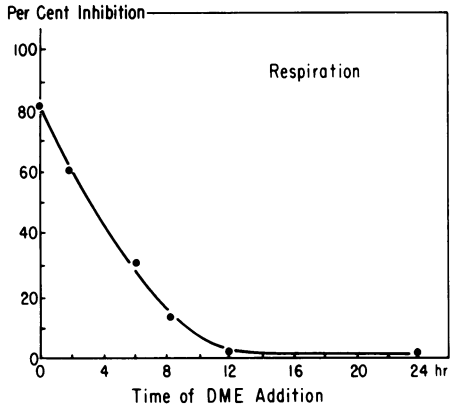

FIG. 3. Effect of time of addition of 10 mM dimethylaminoethanol on the respiratory rise. DME was added after 0, 2, 6, 8, or 12 hr of incubation. Oxygen uptake was measured at the end of the 24-hr interval.

respiration within the first 11 hr (Fig. 3). Application of DME after 11 hr of aging was without effect.

Effect of DME on the Phospholipid Composition of Aged Potato Disks. Lipid extracts of DME-treated tissue run on Silica Gel H thin layer plates showed a spot containing phosphorous that was absent in control samples. The R_F of the unique band from DME-treated tissue corresponds to the phospholipid PDME. Comparative analysis of lipid phosphorous from TLC plates shows that levels of phosphatidylcholine and phosphatidylethanolamine are substantially lower in disks aged in the presence of DME. However, PDME, which is undetectable in control material, comprises almost a third of the phospholipid present in tissue aged in DME for 24 hr (Table VII). Two hr after slicing, lipid extracts from disks held in DME showed traces of PDME upon visual inspection of the TLC plates treated with iodine vapor.

Effect of DME on Fatty Acid Synthesis and Composition. The incorporation of [1-^{14}C]acetate into fatty acids during the first 2-hr pulse after slicing did not differ significantly between DME-treated and control samples.

Analytical gas chromatography of fatty acid methyl esters from aged disks revealed that the total amount of fatty acid synthesized over the 24-hr period in the presence of DME did not differ significantly from that of the aged control. The fatty acid composition of potato disks aged in DME was similar qualitatively to control disks.

Effect of DME on [1-^{14}C]Acetate Incorporation into the Nonsaponifiable Lipid Fraction. The pattern of incorporation of labeled acetate into the nonsaponifiable fraction was different from that observed for the fatty acid fraction of the total lipid extract from potato disks. When DME was added at zero time and the slices were pulsed at 2-hr intervals during aging, label incorporation was consistently inhibited from 30 to 40% compared with that of the control (Table VIII), similar to the case in mammalian cells (7).

Effect of DME on Protein Synthesis. To rule out the possibility that DME had any outright inhibitory effect on protein synthesis, the incorporation of radioactive L-leucine into protein was ascertained in the presence of DME. Disks which were pulsed with leucine and DME for a 2-hr time interval showed no significant inhibition of amino acid incorporation into protein (Table IX).

Disks aged in the presence of DME for longer periods showed some inhibition of protein synthesis. However, the total inhibition over a 24-hr period amounted to only 17 to 20% compared with control samples (Table IX), which limited inhibition may have a rational explanation (see "Discussion").

Table VII. The Effect of DME on the Phospholipid Composition of Potato Disks

Potato disks were aged for 24 hr in the presence of N,N-dimethylethanolamine (DME)[1]. Lipids were extracted and assayed for lipid-phosphorus.

Treatment	Total Lipid Phosphorus	Phosphatidylcholine	Phosphatidyl dimethylethanolamine	Phosphatidylethanolamine	Phosphatidylinositol
			ug phosphorus/g fresh wt.		
Control	13.6	9.9	-	4.5	0.9
DME (1 mM)	12.6	6.0	3.4	2.3	0.9

[1] Effective DME concentration depends on storage history of the tubers.

Table VIII. The Effect of Dimethylaminoethanol on the Incorporation of (1-^{14}C)Acetate into Fatty Acid and Nonsaponifiable Fractions of Potato Disks during the Course of Aging

Tissue disks were treated initially with dimethylaminoethanol (10 mM) and pulsed for 2 hr with (1-^{14}C)acetate (2 uCi/ml) during different intervals of the aging process.

Time	Treatment	Fatty Acid Fraction	Nonsaponifiable Fraction
hr		cpm	
0 - 2	Control	4960	513
	DME	4700	306
5 - 7	Control	8628	415
	DME	6647	278
10-12	Control	11555	1699
	DME	11550	902
22-24	Control	11557	907
	DME	12639	708

Table IX. The Effect of DME on Protein Synthesis

The effect of DME on protein synthesis of potato disks pulsed for 2 hr with L-(U-^{14}C)leucine (0.5 uCi/ml).

Treatment	Incorporation into Protein	Inhibition
	cpm	%
Control	7093	0
DME 1 mM (5 hr)[1]	7906	0
DME 10 mM (5 hr)[1]	7724	0
Control (24 hr)	58897	0
DME 1 mM (24 hr)[2]	48885	17
DME 10 mM (24 hr)[2]	47340	20

[1] Disks pulsed simultaneously with DME and L-(U-^{14}C)leucine during the 5 to 7 hr interval after slicing.

[2] Disks aged in DME for 24 hr after slicing then pulsed for 2 hr with L-(U-^{14}C)leucine.

DISCUSSION

Dependence of the Development of Wound-induced Respiration on Fatty Acid Synthesis. Cerulenin, an antibiotic which curtails *de novo* fatty acid synthesis by irreversible covalent binding to β-ketoacyl-acyl carrier protein synthetase (3), has been shown to be an effective inhibitor of fatty acid synthesis in yeast (15), bacteria (3), animals (19), and plant tissue (P. K. Stumpf, personal communication). We have shown cerulenin to be operative in potato disks as demonstrated by the impairment of radioactive acetate incorporation into lipids and specifically, fatty acids. A limited effect of cerulenin on sterol synthesis in potato slices can be shown, of the level of inhibition reported for yeast (15).

Disks incubated in cerulenin for 24 hr after cutting showed a lower fatty acid content than fresh material, which together with acetate incorporation data indicates that cerulenin blocks fatty acid synthesis while permitting fatty acid degradation. Whereas normal aging leads to an enrichment of 18:1, 18:2, and 18:3 fatty acids, aging in cerulenin leads to no difference in fatty acid composition from that of fresh tissue. While the possibility of a direct effect of cerulenin on the uptake of phospholipid precursors cannot be ignored (*e.g.* Fig. 2), its influence on fatty acid synthesis *per se* (Table IV) suggests that its effect on uptake is indirect, and reflects its influence on fatty acid biosynthesis.

Most important for the main thrust of this investigation, cerulenin inhibits the development of the induced respiration in a time-restricted manner. Whereas cerulenin inhibits respiratory development through a 24-hr period only when given in the first 8 to 10 hr, the inhibition of fatty acid synthesis *per se* by cerulenin persists undiminished through the full 24-hr interval. Thus, with respect to respiratory development, fatty acid synthesis in the first 8 to 10 hr after cutting plays a special role related to the synthesis of phospholipid, and presumably of particular membranes.

The respiration of cerulenin-aged disks remains at levels only slightly higher than that observed for freshly cut material. Furthermore, disks aged in cerulenin remain cyanide-sensitive, a condition characteristic of fresh slices and sharply distinguished from the cyanide insensitivity of aged slices.

The slight effect of cerulenin on protein synthesis may well be indirect, and may be due to the curtailed synthesis of ER membranes as a result of the diminished production of fatty acids for membrane phospholipid. Indirect interference with protein synthesis has been reported for bacteria (8) and yeast (14). Similarly, the low level of inhibition of choline incorporation into phosphatidylcholine by cerulenin represents a secondary effect stemming from the primary inhibition of *de novo* fatty acid synthesis.

Specific Phospholipid Synthesis as an Element in the Development of Induced Respiration. Dimethylaminoethanol interferes with the initiation and development of the induced respiration much as do cerulenin and actinomycin (2). That is to say, its effect is time-restricted. DME can serve as an acceptable substrate for choline kinase (22). The base is phosphorylated and incorporated into cellular phospholipids with ensuing polar head groups different from the normal. Since the amino alcohol is not known to be an enzyme inhibitor *per se*, the accumulation of the DME phosphatidyl derivative in almost one-third of the potato phospholipids in aging slices is considered the causative factor in the curtailment of the developed respiration with age. Tissue aged in DME shows decreased amounts of phosphatidylcholine and phosphatidylethanolamine, indicating that DME serves as an analogue of both bases. Interestingly, there is little difference in the total lipid P between treated and control samples. Thus, DME does not inhibit membrane synthesis, but rather alters the phospholipid composition, which in turn affects the respiratory capacity of the tissue. Examination of membrane fragments from density gradients shows that phosphatidyl DME is integrated into both ER and mitochondrial membranes.

Whereas the suppression of the respiration rise with DME may not be as complete as with actinomycin, the respiration of DME-aged slices is strongly inhibited by cyanide, a condition normally associated only with fresh slices. This suggests that cyanide resistance, which characterizes aged slices, is dependent on the synthesis of specific phospholipids during the course of aging.

In the presence of DME, the total fatty acid composition of aged tissue is approximately the same as that of the aged control. Thus, the major change effected by DME is a change in polar head groups. Ladbrooke and Chapman (11) have pointed out that there is at least a 30-degree difference in the transition temperature from the gel to liquid crystalline phase for phosphatidylcholine and phosphatidylethanolamine, respectively, containing the same acyl side chains—suggesting that the polar head groups may have a role in determining membrane fluidity. Whereas protein synthesis is somewhat inhibited in DME-treated disks, the effect is presumably indirect as previously suggested for cerulenin, and not great enough to account for the extent to which the respiratory rise and cyanide insensitivity are impaired. In short, phosphatidyl DME incorporated in membranes seemingly renders them less functional in sustaining the induced respiration.

DME has a limited effect on the incorporation of radioactive acetate into the nonsaponifiable lipid fraction, again perhaps for indirect reasons. Thus, sterol metabolism may be inhibited by the choline analogue as appears to be the case in animal cells grown in DME, where the levels of desmosterol are lowered (7).

Sterol synthesis does not seem to be a critical element in the development of the induced respiration, however, albeit there is considerable sterol synthesis with aging in potato slices (9). Neither cholesterol nor β-sitosterol alters the effect of cerulenin on the developed respiration. Furthermore, AMO 1618 (2-isopropyl-4-dimethylamino-5-methyl phenyl-1-piperidine carboxylate methyl chloride), an inhibitor of sterol synthesis in plants (4), was administered to aging slices with no effect, as was 20,26-diazocholesterol, an inhibitor of the reduction of desmosterol to cholesterol (16).

In summary, experiments with cerulenin and DME implicate phospholipid biosynthesis as a significant element in the genesis of wound-induced respiration. The time-restricted feature of cerulenin and DME effectiveness place phospholipid, and presumably membrane, biosynthesis as central to the phenomenon of wound-induced respiratory development. Perhaps the foregoing deduction is to be expected since slicing leads to such rapid and pronounced lipid degradation. It remains to be determined whether lipid breakdown products serve as effectors or derepressors in initiating the wound-related response.

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