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Biological Significance of Compartmentation of Hepatic Ethanol Oxidation

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BERRY, M. N., A. R. GRIVELL AND P. G. WALLACE. *Biological significance of compartmentation of hepatic ethanol oxidation.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 201–207, 1983.—Intact cell preparations, isolated from the livers of fasted rats and treated with rotenone or antimycin A, retain a limited ability to oxidize long chain fatty acids but not short chain species or other usual substrates. Cells prepared from livers of animals treated with clofibrate to induce peroxisomal proliferation have even greater long chain fatty acid oxidizing capacity in the presence of these inhibitors. We infer that the peroxisomes of intact cells are capable of catabolizing long chain fatty acids by a superoxide-generating pathway. In normal cells or those from clofibrate-treated rats, reducing equivalents generated within peroxisomes compete with those originating in the cytosol for mitochondrial disposition. Because of this, peroxisomal fatty acid metabolism inhibits ethanol oxidation. Peroxisomal oxidations appear to be coupled to conversation of free energy and mitochondrial-peroxisomal relationships are regulated by interaction of free-energy transducing processes. Hence, uncoupling agents release the inhibition of ethanol oxidation induced by long chain fatty acid. When mitochondrial metabolism is impaired, reducing equivalents may flow from mitochondria to peroxisomes for reaction with O_2. Thus, there exists a two-way interaction between these organelles. The biological and pathological implications of these relationships for ethanol oxidation and overall energy metabolism are discussed.

Ethanol metabolism Peroxisomes Superoxide Intercompartmental interactions Liver
Energy transduction Reversed electron transfer Fatty acid oxidation Fatty acid synthesis

DESPITE many years of intensive effort, the question of how ethanol damages the liver cell remains incompletely resolved [21, 30, 32]. Indeed, the normal metabolism of ethanol is still a matter for debate, particularly in relation to the amount of ethanol catabolized by the various available pathways [5, 24, 29, 41]. Nevertheless, support appears to be gathering for the view that ethanol-induced liver damage involves the production of free-radicals [14, 27, 34]. This proposal is in keeping with the increasing interest in the possibility that spurious free-radical production is a major shared feature underlying many ischemic, degenerative or proliferative processes [13, 19, 20]. The chemical species that has attracted most attention as a putative initiator of free-radical damage is superoxide (O_2^-) [39]. Even in this area, however, there is considerable controversy, with one school of thought arguing that superoxide is a relatively innocuous substance [15,39]. Notwithstanding this, the majority of workers in the field envisage superoxide in combination with H_2O_2 and metal ion catalysts as being a potent source of $OH^\cdot$ and other radicals of high reactivity, capable of causing severe lipid membrane peroxidation and diverse forms of cellular damage [13, 18, 20].

In an aqueous medium superoxide rapidly abstracts protons from H_2O to form $HO_2^\cdot$, which readily undergoes disproportionation to $H_2O_2 + O_2$ [39]. This reaction is accelerated many orders of magnitude in biological systems by the widely distributed superoxide dismutases [17]. However, superoxide in aprotic solvents can also act as a one-electron reductant [39]. These chemical properties of superoxide suggested to us the possibility that it might be a normal and even an essential metabolite involved in cellular energy transduction. Further, we speculated that ethanol might increase the operation of superoxide-generating pathways. Evidence in support of these proposals is provided in this paper.

METHOD

Isolated liver cells from Hooded Wistar rats (250–280 g body wt), starved for 24 hr to deplete liver glycogen, were prepared by a modification of the method of Berry and Friend [2]. The cells (80–120 mg wet wt) were incubated at 37°C in 2 ml of a balanced bicarbonate-saline medium [3] containing albumin, 2.5% w/v, with a gas phase of 95% O_2, 5% CO_2. Consumption of Os measured in the presence of CO_2 by a manometric method [22]. Metabolites were measured by standard enzymic techniques as in [1], on neutralized perchloric acid extracts of the incubated cells. The amount of ethanol oxidized was determined by a radioactive technique [3]. Sources of reagents are described elsewhere [3,7]. 2-(p-Chlorophenoxy)-2-methylproprionic acid ethyl ester (Clofibrate) was administered intramuscularly in a dose of 0.1 g/kg body wt for 14–21 days.

TABLE 1

EFFECTS OF ROTENONE AND ANTIMYCIN A ON METABOLISM OF ISOLATED LIVER CELLS FROM NORMAL AND CLOFIBRATE-TREATED RATS

Treatment	Added* Substrate	O_2-Uptake†			Ketone Bodies			Substrate Uptake		
		Control	Rot‡	Anti‡	Control	Rot	Anti	Control	Rot	Anti
None	None	2.62	1.20	1.07	0.70	0.59	0.16	—	—	—
None	Palmitate	3.40	1.57	1.85	1.66	0.62	0.60	—	—	—
None	Hexanoate	3.56	1.08	0.92	1.99	0.60	0.20	—	—	—
None	Pyruvate	3.39	1.00	0.90	—	—	—	3.61	0.95	1.07
None	Lactate	3.59	1.21	0.95	—	—	—	1.62	0.25	0.32
None	Sorbitol	3.01	1.15	1.00	—	—	—	1.86	0.77	0.18
Clofibrate	None	2.51	1.40	0.90	0.94	0.85	0.18	—	—	—
Clofibrate	Palmitate	3.85	1.48	1.61	2.36	0.57	0.68	—	—	—
Clofibrate	Hexanoate	3.09	1.60	0.75	1.77	0.90	0.13	—	—	—

*Palmitate (2 mM); hexanoate (4 mM); other substrates (10 mM).

†Metabolic rates are expressed as μmol g wet wt/min. Results represent the mean of at least 4 experiments. Standard errors have been excluded for clarity. Incubation time, 40 min.

‡Rotenone (4 μM); antimycin A (1 μm).

RESULTS AND DISCUSSION

The capacity of mammalian peroxisomes for long or medium but not short chain fatty acid oxidation was discovered some 6 years ago [10, 23, 25], but whether they function *in vivo* to catabolize fatty acids has been a matter of considerble argument [10, 16, 23, 25, 31, 35, 42]. Recently, we have obtained compelling evidence that peroxisomal fatty acid β-oxidation plays an important part in fatty acid catabolism by liver cells from starved rats. The full details of this work are published elsewhere [7], but the salient features on which our conclusions are based are recorded in Table 1. The respiratory inhibitors rotenone and antimycin A, each depress the endogenous O_2-consumption of whole cells only about 50–60% at inhibitor levels which virtually abolish the respiration of isolated mitochondria (Table 1). The residual O_2-uptake is associated with accumulation of substantial quantities of ketone bodies, particularly in the presence of rotenone. When long chain fatty acid, as exemplified by palmitate, is provided as an added substrate, respiration is stimulated to some extent despite the presence of inhibitors, with an increment in ketone body production over endogenous rates observed with antimycin A. Short chain fatty acids fail to stimulate O_2-uptake or enhance ketone body formation. That this difference is due to inhibition of mitochondrial oxidations and not to some anomaly of mitochondrial short chain fatty acid "activation" is shown by the strong inhibition of pyruvate oxidation by rotenone or antimycin A (Table 1).

The metabolism of substrates, oxidized in the "cytosolic" compartment, that feed reducing equivalents into the mitochondria via NAD^+-linked shuttles [9] (e.g., lactate) is strongly inhibited by rotenone and antimycin A, while the oxidation of a substrate which can utilize the α-glycerophosphate shuttle [38] (e.g., sorbitol) is inhibited less by rotenone but greatly depressed by antimycin A. Hence it appears that reducing equivalents arising from long chain fatty acid oxidation can be metabolized by rotenone- or antimycin A-treated cells, whereas reducing equivalents derived from other cytosolic or mitochondrial oxidations cannot. In view of the known sites of action of the two respiratory inhibitors [43], it does not seem feasible that the degree of inhibition that they exert could be affected by the nature of the substrate from which the reducing equivalents are derived. We conclude that the oxidation of palmitate and other long chain fatty acids under these circumstances reflects peroxisomal catabolism, a pathway not directly sensitive to rotenone or antimycin A [23,25]. Differences between the metabolism of long and short chain fatty acids are considerably exaggerated in uninhibited and antimycin A-treated cells from rats in which proliferation of hepatic peroxisomes has been induced with clofibrate (Table 1), an observation that provides further support for our conclusion.

We have also shown that added acetoacetate stimulates cellular respiration and increases production of ketone bodies from long and medium but not short chain fatty acids in the presence of rotenone [7]. Moreover, inhibitors of palmitoylcarnitine transferase depress the stimulation of fatty acid oxidation induced by acetoacetate. These observations imply mitochondrial involvement in the additional ketone body formation (since palmitoylcarnitine transferase is exclusively an enzyme of mitochondrial long chain fatty acid metabolism [8,42]). A plausible explanation is that peroxisomes and mitochondria function in concert [26,35,42], peroxisomes initiating fatty acid oxidation and shuttling shorter chain acyl intermediates to the mitochondria where they can be further degraded.

It is apparent also from our studies with rotenone-treated cells (Table 1, see also [7]), in which more than 90% of the ketone bodies accumulate as 3-hydroxybutyrate, that peroxisomes must be capable of transferring reducing equivalents to the mitochondria to bring about the reduction of acetoacetate. Since it requires two turns of the β-oxidation spiral to generate one equivalent of acetoacetate, twice as much NADH is generated as can be removed in 3-hydroxybutyrate. It follows that half the NADH formed in the peroxisomes must be oxidized at a rotenone-insensitive site. Hepatic peroxisomes contain α-glycerophosphate dehydrogenase [42], and it has been postulated that the α-glycerophosphate shuttle serves in these organelles to transfer reducing equivalents to the mitochondria [42]. This pathway would of course be insensitive to rotenone. More-

TABLE 2

LACTATE-STIMULATED ETHANOL OXIDATION IN HEPATOCYTES FROM STARVED NORMAL OR CLOFIBRATE-TREATED RATS

Treatment	Additions*	Metabolic Changes (μmol/g wet wt/min)			
		O_2-Uptake	Ethanol Removed	Acetoacetate	3-Hydroxybutyrate
None	None	3.83 ± 0.21†	1.83 ± 0.10	0.13 ± 0.03	0.26 ± 0.06
None	Hexanoate	5.31 ± 0.31	1.85 ± 0.09	0.41 ± 0.06	1.10 ± 0.08
None	Palmitate	4.62 ± 0.21	1.48 ± 0.11	0.19 ± 0.02	0.83 ± 0.07
Clofibrate	None	4.18 ± 0.17	2.12 ± 0.08	0.16 ± 0.03	0.36 ± 0.09
Clofibrate	Hexanoate	4.72 ± 0.26	2.04 ± 0.20	0.29 ± 0.02	1.31 ± 0.08
Clofibrate	Palmitate	4.76 ± 0.29	1.19 ± 0.10	0.26 ± 0.02	1.54 ± 0.07
Clofibrate	Palmitate, Acetoacetate	5.37 (3)‡	1.42 (3)	−1.79 (3)	3.26 (3)
Clofibrate	Palmitate, NH_4Cl	4.70 ± 0.19	1.96 ± 0.23	0.70 ± 0.16	1.16 ± 0.21
Clofibrate	DNP	6.28 ± 0.39	2.38 ± 0.17	0.47 (4)	0.28 (4)
Clofibrate	Palmitate, DNP	7.97 ± 0.42	2.58 ± 0.14	0.96 ± 0.07	0.64 ± 0.05
Clofibrate	Bromo-octanoate	—	0.60	0.20	0
Clofibrate	Bromo-palmitate	—	0.93	0.15	0.04

*Lactate (10 mM); ethanol (8 mM); palmitate (2 mM); hexanoate (4 mM); NA_4Cl (10 mM); acetoacetate (10 mM); DNP (50 μM, albumin-bound, free concentration, circa 5 μM).

†Except where indicated, results are means ± S.E.M. of at least 10 experiments. Incubation time, 40 min.

‡Number of experiments in parentheses.

over, hepatic peroxisomes are devoid of malate dehydrogenase [42] and we have found that fatty acid oxidation is insensitive to fluoromalate, a potent inhibitor of this enzyme [7].

The picture emerges of a shuttle system whereby half the long chain hydroxyacyl CoA in cells exposed to rotenone is oxidized within the peroxisomes, the reducing equivalents being transferred to the mitochondria in α-glycerophosphate. The remaining hydroxyacyl CoA can pass to the mitochondria and provide reducing equivalents for acetoacetate reduction through a coupled redox reaction catalyzed by mitochondrial hydroxyacyl CoA dehydrogenase and 3-hydroxybutyrate dehydrogenase. The remarkable degree of interaction between peroxisomes and mitochondria can be further appreciated from the observation that antimycin A (which will block mitochondrial flavin-linked oxidations) does not cause additional inhibition of palmitate oxidation when added to cells incubated with rotenone (data not shown). This implies that reducing equivalents generated during the mitochondrial oxidation of long chain acyl CoA can pass back to the peroxisomes for disposal via the antimycin A-insensitive superoxide-producing pathway. Hence, reducing equivalents can flow in either direction between the two organelles.

Reducing-Equivalent Transfer Between Cell Compartments

When the [lactate]/[pyruvate] ratio is elevated by addition of 10 mM lactate to normal cells, it gradually returns to normal (circa 8/1) over a period of some 40 min [6]. This restoration to normal steady-state is much slower in the presence of palmitate than when hexanoate is added [7]. Likewise, lactate-stimulated ethanol oxidation [12] is substantially depressed by added palmitate though not by hexanoate (Table 2). These differences in behavior between palmitate and hexanoate again suggest peroxisomal involvement in long chain fatty acid oxidation and a competition for transfer to the mitochondria between reducing equivalents arising in the cytosol and those generated within the peroxisomes.

Supporting evidence for this comes from studies with cells from clofibrate-treated animals in which palmitate depresses lactate-stimulated ethanol oxidation still further (Table 2). Moreover, in cells from animals receiving twice the usual dose of clofibrate, glucose synthesis from lactate, although occurring at normal rates in the absence of palmitate, is strongly inhibited by the added long chain fatty acid (though not by hexanoate). Under these circumstances the [lactate]/[pyruvate] ratio remains very high whereas it falls towards normal in the presence of hexanoate. Both ethanol and lactate oxidation can be restored by addition of an artificial electron carrier such as methylene blue, indicating that the depression induced by palmitate is not a result of direct enzyme inhibition (data not shown).

In normal cells and in cells from clofibrate-treated animals, palmitate and hexanoate enhance the reduction of added pyruvate to lactate to the same extent (unpublished observations; see also [4]). Hence, export of reducing equivalents from the mitochondria is similar for each fatty acid. In rotenone-treated cells, however, pyruvate reduction is depressed considerably even in the presence of added fatty acid (Table 3), although the coupling of the redox reactions catalyzed by hydroxyacyl CoA dehydrogenase and lactate dehydrogenase should be thermodynamically favorable [4]. Addition of acetoacetate partially restores pyruvate reduction to lactate when palmitate is present, but not in the absence of added long chain fatty acid. These data show that reducing equivalents generated within the peroxisomes cannot couple directly with cytosolic dehydrogenases so that reducing equivalents for NAD^+-linked cytosolic reductions must be derived from the mitochondria. This is a particularly

TABLE 3

EFFECTS OF ROTENONE AND ANTIMYCIN A ON PYRUVATE REDUCTION OR LACTATE OXIDATION IN NORMAL LIVER CELLS

Added Substrates*	Other Additions			Metabolic Changes† (μmol/g wet wt/min)		
	Rot‡	Anti§	AcAc¶	O_2-Uptake	Lactate	[Lactate]/[Pyruvate]
Pyruvate	–	–	–	3.39	0.89	—
Pyruvate	+	–	–	0.95	0.41	—
Pyruvate	+	–	+	0.88	0.45	—
Pyruvate	–	+	–	1.01	0.37	—
Lactate	–	–	–	3.60	−1.88	10.8
Lactate	+	–	–	1.25	−0.31	109
Lactate	+	–	+	1.19	−0.33	270
Lactate	–	+	–	1.08	−0.52	483
Palmitate, Pyruvate	–	–	–	4.31	1.20	—
Palmitate, Pyruvate	+	–	–	2.10	0.40	—
Palmitate, Pyruvate	+	–	+	3.40	0.81	—
Palmitate, Pyruvate	–	+	–	2.48	0.76	—
Palmitate, Lactate	–	–	–	4.93	−2.40	10.4
Palmitate, Lactate	+	–	–	1.50	−0.50	78.2
Palmitate, Lactate	+	–	+	3.15	−0.59	25.6
Palmitate, Lactate	–	+	–	2.20	−1.18	51

*Pyruvate (10 mM); lactate (10 mM); palmitate (2 mM).
†Incubation time, 40 min. Results are the means of 3–10 experiments.
‡Rotenone (4 mM).
§Antimycin A (1μM).
¶Acetoacetate (8 mM).

important observation because it indicates that the competition between cytosolic and peroxisomal reducing equivalents for mitochondrial respiratory components takes place at the level of the mitochondrion itself.

This proposal can be tested by addition of agents which can bring about oxidation of the mitochondrial NADH pool, e.g., acetoacetate, ammonium chloride and the uncoupler, 2,4-dinitrophenol (DNP). The data of Table 2 show that both acetoacetate and ammonium chloride relieve to some extent the inhibition of lactate-stimulated ethanol oxidation by palmitate, but DNP is considerably more effective, causing a complete release of inhibition. From this observation we infer that the inhibition of ethanol oxidation by peroxisomal catabolism of palmitate is due to competition for the energy transducing pathways of the mitochondrial respiratory chain distal to NADH dehydrogenase. This can be explained if, as postulated [42], reducing equivalents arising from palmitate oxidation within the peroxisomes are transferred to the mitochondria via the α-glycerophosphate shuttle and enter the mitochondrial energy transducing system at the level of flavoprotein.

Of particular interest is the finding that in the presence of DNP the rate of ethanol oxidation is actually enhanced by added palmitate (Table 2). This led us to suspect that a cyclical process might be established whereby palmitate catabolism in the peroxisomes would provide acetyl CoA for chain elongation [36] in the mitochondria, with ethanol (or acetaldehyde) contributing reducing equivalents (and perhaps C_2 fragments [28]). Support for the possibility that ethanol oxidation might in this way be obligatorily linked to hepatic fatty acid turnover was provided by experiments that

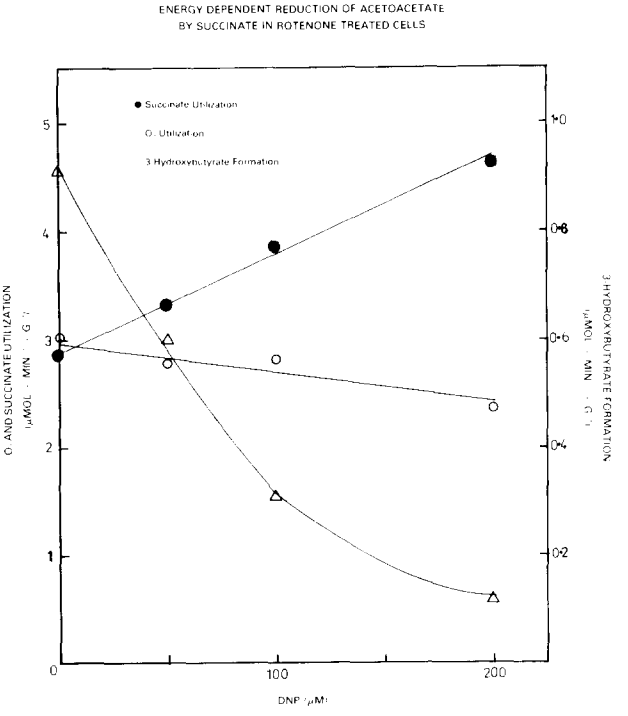

FIG. 1. Energy-dependent reduction of added acetoacetate by isolated liver cells incubated in the presence of succinate, palmitate and rotenone. Incubation time was 40 min.

show a strong depression of ethanol oxidation by the potent inhibitors of fatty acid metabolism, bromo-palmitate and bromo-octanoate (Table 2). The process of chain elongation could readily account for the free-energy consumption suggested by the very high O_2-uptake under circumstances where there appears to be minimal demand for ATP [12,41].

Energy Transduction Within Peroxisomes

Further examination of peroxisomal-mitochondrial interactions provided persuasive evidence that peroxisomes are energy-transducing organelles in their own right. We have previously shown that palmitate (but not hexanoate) can restore the [lactate]/[pyruvate] ratio of rotenone-poisoned cells when acetoacetate is present [4,6] (see also Table 3). Palmitate can also drive pyruvate reduction under these circumstances [4]. This was explained on the basis of restoration by acetoacetate of energy transducing pathways beyond the rotenone block, the inference being that the energy transducing system was contained entirely within the mitochondrion. Unexpectedly, we observed that pyruvate reduction and restoration of the [lactate]/[pyruvate] ratio, perturbed by a lactate load, could be achieved in the presence of antimycin A, even without addition of acetoacetate (although gluconeogenesis from lactate remained virtually completely inhibited). Since the correction of the [lactate]/[pyruvate] ratio is an energy-dependent process involving mitochondrial-cytosolic interactions [4, 6, 9], we were faced with the startling conclusion that energy-linked process within the mitochondria could be driven by peroxisomal metabolism.

The utilization of free energy generated within the peroxisomes to drive mitochondrial energy-dependent processes seemed so unlikely that we felt it necessary to undertake rigorous further testing. We used antimycin A-treated cells in which succinate oxidation was markedly inhibited (Table 4). This inhibition could be released by N,N,N′,N′,-tetramethyl-p-phenylenediamine (TMPD), and the TMPD bypass of the antimycin A site [43] could in turn be blocked by ascorbate [43]. When palmitate (but not hexanoate) was added to antimycin A-treated cells incubated with succinate (in the absence of TMPD) there was a stimulation of O_2-consumption of over 1 μmol/g wet wt/min. Balance studies showed that more than 50% of the additional O_2-uptake could be attributed to succinate oxidation to malate and fumarate. This stimulation of antimycin A-inhibited succinate oxidation by added palmitate was unaffected by ascorbate (even in the presence of TMPD), but was inhibited by DNP. The conclusion seems inescapable that the peroxisomal oxidation of palmitate was driving the mitochondrial oxidation of succinate, the reducing equivalents being shuttled via a mitochondrial NAD^+ pool to the peroxisomes. The presence of rotenone in addition to antimycin A did not inhibit the pathway, providing evidence that the peroxisomal flavin-linked superoxide-producing reaction could be the free-energy generating step. When added acetoacetate was present, it was reduced to 3-hydroxybutyrate by succinate in a DNP-sensitive manner (Fig. 1), substantiating our conclusion that flavin-linked oxidations within hepatic peroxisomes can drive mitochondrial energy-dependent reversed electron transfer [11].

Conclusions

Until now it has been generally believed that peroxisomes have no energy-transducing functions and that peroxisomal oxidations are merely heat producing processes. These conclusions have been based, in the main, on the inability to demonstrate conservation of free energy within the isolated organelle [23, 25, 42] and on the lack of ATP-synthetase in its boundary membrane [42]. However, it is well-recognized that broken cell preparations do not necessarily possess all the properties of the intact system. Moreover, the insights of Mitchell [33] have led to the recognition that the primary free-energy conserving process in membrane-bounded organelles involves the establishment of a proton gradient across the transducing membrane, and that this gradient can be utilized for energy-dependent functions not necessarily directly linked to ATP turnover [40,44]. If, as seems likely, the peroxisomal membrane *in vivo* is competent to maintain such a gradient, it can be anticipated how oxidations involving superoxide production could establish charge separation. The formation of superoxide anion within the membrane of the peroxisome, followed by its extrusion into the matrix, will confer on the membrane a net positive charge. Proton abstraction from matrix H_2O followed by superoxide dismutase-catalysed disproportionation [17] and catalatic or peroxidative removal of H_2O_2 [41] will establish an excess of OH^- ions within the peroxisomal matrix, effectively equivalent to a proton gradient and electric field across the membrane, exterior positive (Fig. 2). We infer that such a gradient could be used to drive energy-dependent reductive syntheses and active ion transport.

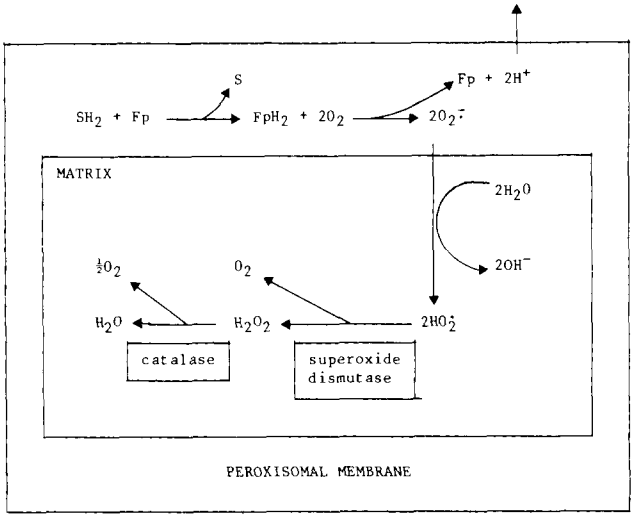

FIG. 2. Putative mechanism for generation of a "proton gradient" across the peroxisomal membrane. The substrate (SH_2)—e.g., acyl CoA—reduces the flavoprotein oxidase (Fp). Electron transfer from FpH_2 to O_2 occurs in two steps, generating an intermediary flavin semiquinone radical.

The implications of these suggestions in relation to overall cellular metabolism are obvious and need not be elucidated here. Clearly, the function of other superoxide-producing systems such as the endoplasmic reticulum and outer mitochondrial membrane require reassessment. The mechanism by which coupling of energy transduction between individual organelles takes place will also need elucidation. An attractive possibility, suggested by our experiments with antimycin A-treated cells, is that under normoxic conditions inner mitochondrial membrane metabolism is favored and superoxide formation accordingly partially suppressed.

TABLE 4

EFFECTS OF PALMITATE ON SUCCINATE OXIDATION BY NORMAL CELLS IN THE PRESENCE OF ANTIMYCIN A

Additions*	Antimycin A†	O_2-Uptake‡	Malate and Fumarate Accumulated
Succinate	–	3.84 ± 0.10	3.05 ± 0.16
Succinate	+	2.31 ± 0.08	1.53 ± 0.19
Succinate, TMPD	+	3.60	2.01
Succinate, Ascorbate	+	—	0.40
Succinate, TMPD, Ascorbate	+	3.59	0.57
Palmitate, Succinate	–	4.98 ± 0.16	2.92 ± 0.38
Palmitate, Succinate	+	3.48 ± 0.29	2.71 ± 0.21
Palmitate, Succinate, Ascorbate	+	—	1.73
Palmitate, Succinate, TMPD, Ascorbate	+	4.70	1.82
Palmitate, Succinate, DNP	+	1.56	0.73
Succinate, Rotenone	–	3.32	3.23
Succinate, Rotenone	+	2.02	1.51
Palmitate, Succinate, Rotenone	+	3.34	3.56

*Succinate (15 mM); palmitate (2 mM); ascorbate (8 mM); TMPD (0.3 mM); rotenone (4 μM).
†Antimycin A (1 μM).
‡Metabolic rates are expressed as μmol/g wet wt/min. The incubation time was 40 min. Results, where standard errors are shown, are the means of at least 4 experiments. Other values are the average of 2 experiments.

Conditions which impair inner mitochondrial membrane function such as hypoxia [21], carbon monoxide intoxication (or antimycin A-treatment) will allow expression of peroxisomal oxidation. Alternatively, particular metabolic fuels (e.g., long chain fatty acids) or drugs, (e.g., ethanol, phenobarbital [37]), or inadequate or abnormal diet [32] may induce superoxide-generating pathways in the peroxisomes and endoplasmic reticulum. The balance of function between the cytochrome oxidase-dependent pathway of the inner mitochondrial membrane and the more primitive superoxide-generating systems of other membranes may well be an important factor determining the incidence of tissue damage.

These studies, although at present rudimentary, should also advance our understanding of the physiological and pathological implications of ethanol metabolism. It seems reasonable to assume that ethanol and acetaldehyde represent two of the most primitive of metabolic fuels, and it is likely that the cells of many tissues possess pathways for their oxidation, involving superoxide formation. Investigation of the mechanisms by which electron flow along such pathways can initiate disease processess should prove a rewarding area for research.

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REFERENCES

1. Bergmeyer, H. U. *Methods of Enzymatic Analysis*, 2nd edition. New York: Academic Press, 1974.
2. Berry, M. N. and D. S. Friend. High-yield preparation of isolated rat liver parenchymal cells. *J Cell Biol* **43:** 506–520, 1969.
3. Berry, M. N., H. V. Werner and E. Kun. Effects of bicarbonate on intercompartmental reducing-equivalent translocation in isolated parenchymal cells from rat liver. *Biochem J* **140:** 355–361, 1974.
4. Berry, M. N. The function of energy-dependent redox reactions in cell metabolism. *FEBS Lett* Suppl. **117:** K106–K120, 1980.
5. Berry, M. N., D. C. Fanning, A. R. Grivell and P. G. Wallace. Ethanol oxidation by isolated hepatocytes from fed and starved rats and from rats exposed to ethanol, phenobarbitone or 3-amino-triazole. *Biochem Pharmacol* **29:** 2161–2168, 1980.
6. Berry, M. N., A. R. Grivell and P. G. Wallace. Energy-dependent regulation of the steady-state concentrations of the components of the lactate dehydrogenase reaction in liver. *FEBS Lett* **119:** 317–322, 1980.
7. Berry, M. N., A. R. Grivell and P. G. Wallace. Compartmentation of fatty acid oxidation in liver cells. *Eur J Biochem*, in press, 1982.
8. Bieber, L. L., J. B. Krahling, P. R. H. Clarke, K. J. Valkner and N. E. Tolbert. Carnitine acyltransferases in rat liver peroxisomes. *Arch Biochem Biophys* **211:** 599–604, 1981.
9. Bremer, J. and E. J. Davis. Studies on the active transfer of reducing equivalents into mitochondria via the malate aspartate shuttle. *Biochim Biophys Acta* **376:** 387–397, 1975.
10. Bronfman, M., N. C. Inestrosa and F. Leighton. Fatty acid oxidation by human liver peroxisomes. *Biochem Biophys Res Commun* **88:** 1030–1036, 1979.
11. Chance, B. and G. Hollunger. The interaction of energy and electron transfer reactions in mitochondria. I. General properties and nature of the products of succinate-linked reduction of pyridine nucleotide. *J Biol Chem* **236:** 1534–1543, 1961.

12. Crow, K. E., N. W. Cornell and R. L. Veech. Lactate-stimulated ethanol oxidation in isolated rat hepatocytes. *Biochem J* **172:** 29–36, 1978.
13. Del Maestro, R. F., H. H. Thaw, J. Björk, M. Planker and K.-E. Arfors. Free radicals as mediators of tissue injury. *Acta Physiol Scand* Suppl. **492:** 43–57, 1980.
14. DiLuzio, N. R. Antioxidants, lipid peroxidation and chemical-induced liver injury. *Fed Proc* **32:** 1875–1881, 1973.
15. Fee, J. A., A. C. Lees, P. L. Bloch, F. C. Neidhardt. The role of superoxide in the induction of superoxide dismutase and oxygen toxicity. In: *Biochemical and Clinical Aspects of Oxygen*, edited by W. S. Caughey and H. Caughey. New York: Academic Press, 1979, pp. 635–658.
16. Foerster, E.-C., T. Fährenkemper, U. Rabe, P. Graf and H. Sies. Peroxisomal fatty acid oxidation as detected by H_2O_2 production in intact perfused rat liver. *Biochem J* **196:** 705–712, 1981.
17. Fridovich, I. Superoxide dismutases. *Ann Rev Biochem* **44:** 147–159, 1975.
18. Fridovich, I. The biology of oxygen radicals. *Science* **201:** 875–880, 1978.
19. Gerschmann, R., D. L. Gilbert, S. W. Nye, P. Dwyer and W. O. Fenn. Oxygen poisoning and x-irradiation. A mechanism in common. *Science* **119:** 623–626, 1954.
20. Halliwell, B. Free radicals, oxygen toxicity and aging. In: *Age Pigments*, edited by R. S. Sohal. New York: Elsevier/North Holland, 1981, pp. 1–62.
21. Israel, Y., H. Kalant, H. Orrego, J. H. Khanna, L. Videla and J. M. Phillips. Experimental alcohol-induced hepatic necrosis: suppression by propylthiouracil. *Proc Natl Acad Sci USA* **72:** 1137–1141, 1975.
22. Krebs, H. A., N. W. Cornell, P. Lund and R. Hems. Some aspects of hepatic energy metabolism. In: *Regulation of Hepatic Metabolism*, edited by F. Lundquist and N. Tygstrup. Copenhagen: Munksgaard, 1974, pp. 726–750.
23. Lazarow, P. B. and C. de Duve. A fatty acyl-CoA oxidizing system in rat liver peroxisomes: enhancement by clofibrate, a hypolipidemic drug. *Proc Natl Acad Sci USA* **73:** 2043–2046, 1976.
24. Lazarow, P. B. Metabolism of H_2O_2 and fatty acids by rat liver peroxisomes: Implications for ethanol detoxification. In: *Alcohol and Aldehyde Metabolizing Systems*, vol. 2, edited by R. G. Thurman. New York: Plenum Press, 1977, pp. 275–289.
25. Lazarow, P. B. Rat liver peroxisomes catalyze the β-oxidation of fatty acids. *J Biol Chem* **253:** 1522–1528, 1978.
26. Lazarow, P. B. Compartmentation of β-oxidation of fatty acids in peroxisomes. In: *Metabolic Compartmentation*, edited by H. Sies. New York: Academic Press, 1982, pp. 317–329.
27. Lewis, K. O. and A. Paton. Could superoxide cause cirrhosis? *Lancet* **2:** 188–189, 1982.
28. Lieber, C. S. and R. Schmid. The effect of ethanol on fatty acid metabolism: Stimulation of hepatic fatty acid synthesis *in vitro*. *J Clin Invest* **40:** 394–399, 1961.
29. Lieber, C. S. and L. M. DeCarli. Hepatic microsomal ethanol-oxidizing system. *J Biol Chem* **245:** 2505–2512, 1970.
30. Lieber, C. S. Alcohol and malnutrition in the pathogenesis of liver disease. JAMA **233:** 1077–1082, 1975.
31. Mannaerts, G. P., L. J. Debeer, J. Thomas and P. J. De Schepper. Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate treated rats. *J Biol Chem* **254:** 4585–4595, 1979.
32. Mezey, E. Alcoholic liver disease: roles of alcohol and malnutrition. *Am J Clin Nutr* **33:** 2709–2718, 1980.
33. Mitchell, P. Vectorial chemistry and the molecular mechanisms of chemiosmotic coupling: power transmission by proticity. *Biochem Soc Trans* **4:** 399–430, 1976.
34. Müller, H. and H. Sies. Role of alcohol dehydrogenase activity and of acetaldehyde in ethanol-induced ethane and pentane production by isolated perfused rat liver. *Biochem J* **206:** 153–156, 1982.
35. Neat, C. E., M. S. Thomassen and H. Osmundsen. Effects of high-fat diets on hepatic fatty acid oxidation in the rat. *Biochem J* **196:** 149–159, 1981.
36. Quagliariello, E., C. Landriscina and P. Coratelli. Fatty acid synthesis by chain elongation in rat liver mitochondria. *Biochim Biophys Acta* **164:** 12–24, 1968.
37. Remmer, H. The induction of hydroxylating enzymes by drugs. FEBS Symp **16:** 125–141, 1969.
38. Sacktor, B. and A. Dick. Pathways of hydrogen transport in the oxidation of extramitochondrial reduced diphosphopyridine nucleotide in flight muscle. *J Biol Chem* **237:** 3259–3263, 1962.
39. Sawyer, D. T. and J. S Valentine. How super is superoxide? *Accounts Chem Res* **14:** 393–400, 1981.
40. Skulachev, V. P. Membrane electricity as a convertible energy currency for the cell. *Can J Biochem* **58:** 161–175, 1980.
41. Thurman, R. G. Hepatic alcohol oxidation and its metabolic liability. *Fed Proc* **36:** 1640–1646, 1977.
42. Tolbert, N. E. Metabolic pathways in peroxisomes and glyoxysomes. *Annu Rev Biochem* **19:** 133–157, 1981.
43. Wainio, W. W. The respiratory chain. In: *The Mammalian Mitochondrial Respiratory Chain*. New York: Academic Press, 1970, pp. 110–125.
44. Wanders, R. J. A., H. E. Van Doorn and J. M. Tager. The energy-linked transhydrogenase in rat liver in relation to the reductive carboxylation of 2-oxoglutarate. *Eur J Biochem* **116:** 609–614, 1981.