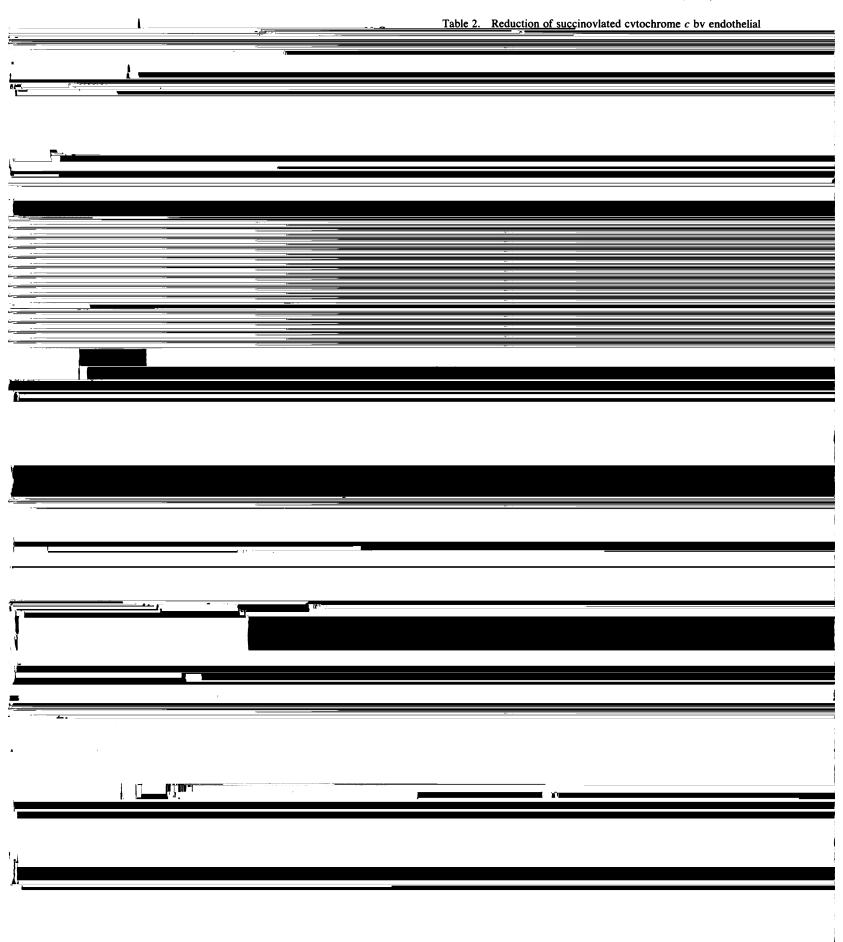
Detection of superoxide generated by endothelial cells (spin trapping/lipid peroxidation) GERALD M. ROSEN*† AND BRUCE A. FREEMAN‡ Departments of *Pharmacology and †Medicine, Duke University Medical Center. Durham. NC 27710: and †GRECC. Veterans Administration Medical Center. Durham, NC 27705 Communicated by Irwin Fridovich. July 3. 1984 ABSTRACT Superoxide and lipid free-radical generation membranes was observed by spin-trapping lipid free radi-

310	sured by superoxide dismutase-inhibitable reduction of 30	le 1. Oxvgen consumption by endothelial cell suspensions
		V
-		
	μ M succinoylated cytochrome c , monitored at 550 nm (ε_{max}	nmol O ₂ consumed
	, · · · · · · · · · · · · · · · · · · ·	
.		
1 1 1 1 1 1 1 1 1 1		
).		
		V
and the second s	(34) 771	
A		
1		
1		
III h		
<u>T</u> j.,		
· -		
<u> </u>		
		V
_		
12 +	·	
, <u>}=</u> ′	, ,	

Biochemistry: Rosen and Freeman

<u></u>	An EPR spectrum characteristic of DMPO-OH (Fig. 1)	vielding an autoxidizable semiquinone (25). Dicoumarol inhi-
	might suggest that hydroxvl radical was produced by mena-	bition of the two-electron reduction of menadione by DT-
<u>.</u>	dione-treated endothelial cells. However, this is probably	diaphorase [NAD(P)H:(quinone-acceptor)oxidoreductase
	not the case. since DMPO-OOH rapidly decomposes into	(EC 1.6.99.2): see ref. 251 diminishes extracellular superox-
-		
1		
<u> </u>		
<u>Na. 2000.</u>	···	
<u>V</u>		
,		
1		
1		
		
1		
ii.		
*		
-		
-		
<i>i</i> -		
<i>f</i> i		
#**		,
<u> </u>		
<i>I</i> —		
	droxyl radical (21, 22). Approximately 3% of DMPO-OH	hvdroxvnaphthalene available for extracellular diffusion and
*		
<i>i</i> -		



Biochemistry: Rosen and Freeman

\$5	droxvnanthalene available for extracellular diffusion (Table	This work was supported in part by American Cancer Society
· · · · · · · · · · · · · · · · · · ·		W- '
Atra.		
F		
,1-		
4		
		•
r	a property and a	
		ļ.
AT U DELL		
1		
I_{ij}		
—		
	·	
1		
•		
<u>.</u>		
	11	
·		**
4		
l _m		
k.		
<u> </u>		
<u> </u>		
		!
		N
<u></u>		
7-4-3-4-6-		
T _{err}		
* <u> </u>	A Married (Karan	
(<u>L</u>		
\ <u></u>		
Mary who was a way	CALLAND.	
<u> </u>		