Effect of a Pseudomonas Fraction on Oxidase Activity of Mitochondria and Monocytes.* (26106)

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Berk *et al.*†(1) indicated that the succinoxidase activity of cells obtained from mouse peritoneal exudates, as well as of homogenates of liver and spleen, was inhibited in presence of heat-killed *Pseudomonas aeruginosa*. Succinoxidase activity was also reduced when these cells (monocytes) were allowed to phagocytize dead Pseudomonas *in vivo*. Cytochrome oxidase activity of monocytes and tissue homogenates seemed to be less inhibited by the presence of dead organisms. Recent data obtained in this laboratory indicate that *in vitro* succinoxidase activity of mouse liver mitochrondria and of monocytes is also depressed by trace amounts of Piromen‡ (a Pseudomonas polysaccharide).

Materials and methods. Mitochondria were prepared by homogenizing livers of 20 mice in chilled 8.5% sucrose-0.1 M phosphate buffer solution, pH 7.3, in a Waring blendor for 60 seconds. Following removal of cellular debris by low speed centrifugation, the homogenates were centrifuged for 20 minutes at 20,000 × g and the sedimented mitochondria resuspended in buffered sucrose solution. Peritoneal exudates (monocytes) were induced in 40 mice by injection of 0.5 ml mineral oil. The cells were harvested 16 hours later, washed, and examined manometrically as previously described(1). Manometric experiments were run in the conventional manner at pH 7.3 and total flask volume was 3.2 ml. The pH of the Piromen was adjusted to 7.3 with Na_2HPO_4 prior to use. Monocytes and mitochondria were incubated respectively with Piromen 30 minutes prior to addition of substrates. The incubation conditions for mitochondria studies were as follows: 20 μmoles of succinate or 50 μmoles of para-phenylenediamine (p-PDA), 0.4 mg N/ml of liver mitochondria, 100 μmoles of phosphate, pH 7.3; 0.5 μmole of cytochrome c, 2 μmoles of $MgCl_2$, 10 μmoles of NaCl. Monocyte experiments were performed with heparinized phosphate buffer, pH 7.3. Substrate and cytochrome levels were the same as in mitochondria experiments. All experiments were at 38°C.

Results. Table I indicates that both succinic and cytochrome oxidase activities of liver mitochondria were inhibited by as little as 3 μg of Piromen per ml. In one preparation, succinoxidase activity was completely abolished by 3 μg of Piromen per ml. It was more typical, however, for inhibition of succinoxidase activity to disappear upon prolonged incubation. Fig. 1 depicts this reversal of inhibition and indicates that the inhibitory effect of Piromen was greatest at beginning of reaction. The data in Table I and Fig. 2 suggest that cytochrome oxidase was more sensitive to Piromen inhibition than was succinoxidase. Although the greatest inhibition of cytochrome oxidase also occurred at beginning of reaction, nevertheless there was less reversal of inhibition than noticed in the succinate system. Since endogenous respiration of mouse liver mitochondria was negligible, it was difficult to deter-

TABLE I. Effect of Piromen on Succinic and Cytochrome Oxidase Activities of Mouse Liver Mitochondria.

Substrate	Enzyme conc., mg N/ml	μl O_2/90 min. Piromen* —	+
Succinic acid	.40	199	175
" "	.40	194	168
" "	.38	184	125
p-PDA	.55	293	216
"	.40	243	170
"	.40	237	168

* 3 μg/ml.

* This investigation supported by grant from Nat. Inst. of Allergy and Infect. Dis., U.S.P.H.S.

† Presented at joint meeting of the Southern Calif. Branch of Soc. Am. Bact. and Western Branch of AAAS, San Diego, June 17, 1959.

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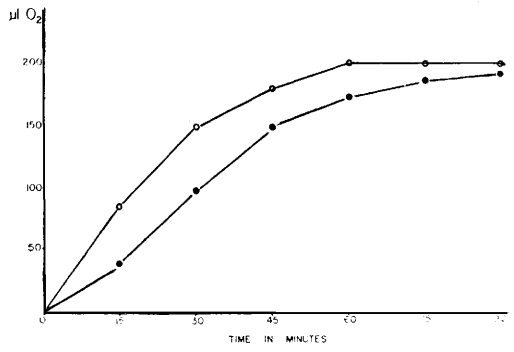

FIG. 1. Effect of Piromen on succinic oxidase activity of liver mitochondria. Open circles represent activity in absence of Piromen; closed circles represent activity in presence of 5 µg of Piromen/ml.

mine whether this activity was sensitive to Piromen.

Kerby(2) previously reported that Piromen disrupts the cell structure of human leukocytes. Therfore, Piromen-treated monocytes were also included in this study. The data in Table II indicate that the succinoxidase activity of mouse monocytes was sensitive to Piromen and degree of inhibition was proportional to concentration of polysaccharide added. However, the cytochrome oxidase activity of several preparations examined appeared to be relatively insensitive to inhibition. Where inhibition was observed with cytochrome oxidase there was no good correlation between degree of inhibition and Piromen concentration. It should be emphasized that the inhibitory effect of Piromen on monocyte succinoxidase activity was not overcome with continued incubation as noted with liver mitochondria. Consequently, inhibition was not rate limiting, but rather suppressive.

TABLE II. Effect of Piromen Concentration on Succinic and Cytochrome Oxidase Activities of Mouse Monocytes.*

Piromen, µg/ml	µl O_2/120 min. Succinate	p-PDA
.0	112	140
.5	110	
1.0	103	149
2.0		143
3.0	86	126
5.0	20	

* 10^7 monocytes/ml.

Since Piromen was inhibitory in trace amounts and thus resembled exotoxins in activity, the question arose as to its heat lability. Table III demonstrates that the ability of a solution of Piromen to inhibit mitochondria and monocytes is not reduced by boiling directly in the flame of a burner for a few seconds.

TABLE III. Comparison of Heated and Unheated Piromen on Succinic and Cytochrome Oxidase Activities of Mouse Cell Preparations.

Preparation	Substrate	µl O_2
Monocytes*	Succinate	177
	" + 5 µg Piromen/ml	21
	" + 5 µg heated Piromen/ml	46
Mitochondria†	p-PDA	346
	" + 3 µg Piromen/ml	262
	" + 3 µg heated Piromen/ml	238

* 10^7 monocytes/ml—180 min. incubation.

† .4 mg N/ml of liver mitochondria—90 min. incubation.

Discussion. Previous studies(1) indicated that the succinoxidase activity of either mitochondria (liver) or intact cells (monocytes, HeLa) could be depressed by heat-killed *Pseudomonas aeruginosa* or sonic fractions thereof. Glycolysis was not inhibited (un-

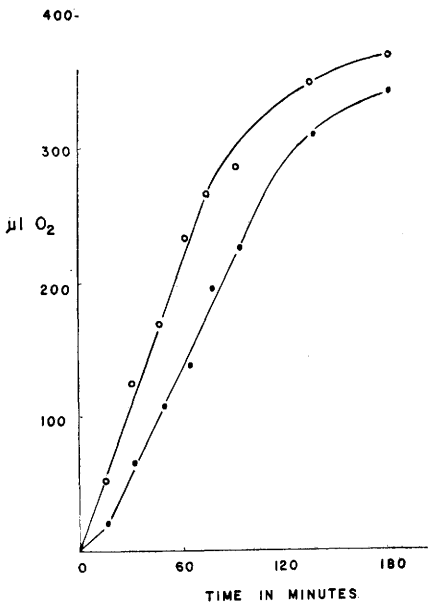

FIG. 2. Effect of Piromen on cytochrome oxidase activity of liver mitochondria. Open circles represent activity in absence of Piromen; closed circles represent activity in presence of 5 µg of Piromen per ml.

published results). In the present study inhibition of both succinic and cytochrome oxidases has been effected with a carbohydrate fraction of *Pseudomonas* (prepared commercially as Piromen). A few micrograms of Piromen were as inhibitory as several hundred micrograms N/ml of intact *P. aeruginosa*, and inhibition was proportional to concentration of Piromen used. With large amounts (5-10 μg) succinoxidase activity of monocytes was completely suppressed, but with lesser amounts only rate of reaction was affected.

Although both succinic and cytochrome oxidases were inhibited by Piromen, type and degree of inhibition was not constant for all tissues. In mouse monocytes succinoxidase activity could be reduced or completely abolished in proportion to amount of drug added. Once inhibited, little or no recovery of the system occurred. The cytochrome oxidase of monocytes was essentially resistant to inhibition. On the other hand, in liver mitochondria the succinoxidase system was able to overcome an initial inhibitory effect of Piromen, while the cytochrome oxidase system was more sensitive to inhibition by the drug. The fact that trace amounts of Piromen are toxic to cells and that the toxicity is heat stable suggests that Piromen possesses properties common to both exo and endotoxins.

The observation that mammalian oxidase activity is inhibited by trace amounts of a bacterial polysaccharide resembles that of Ajl(3) and Packer *et al.*(4). These investigators noted that alpha-keto glutarate oxidation in crude mouse liver homogenates and preparations of rat heart sarcosomes was inhibited by *Pasteurella pestis* toxin.

Piromen has been reported to be a nonprotein polysaccharide complex containing lipid and nucleic acids(5). It, therefore, differs chemically from Pasteurella toxin. Whether at the molecular level the active portions of the Pseudomonas and Pasteurella inhibitors differ or are very similar remains to be determined. In any case, some progress has been made toward determining the site of metabolic interaction between these parasites and their hosts.

In a related study, Gelzer and Suter(6) have reported that virulent strains of some bacteria, when phagocytized, inhibit endogenous respiration of rabbit leucocytes, whereas avirulent strains do not. The difference between virulence and avirulence may well be related to chemical differences in surface components (antigens) of the bacteria. Apparently bacteria differ with respect to site of their action on host cell metabolism, and although Pseudomonas and Pasteurella affect oxidative reactions, preliminary experiments with fractions of other organisms such as *Malleomyces pseudomallei, Vibrio fetus, Escherichia coli,*§ *Serratia marcescens,*§ and Salmonella typhose§ indicate no such activity.

Summary. A commercially prepared polysaccharide (Piromen) from a species of *Pseudomonas* inhibited both succinic and cytochrome oxidases of mouse liver mitochondria *in vitro*. Piromen alters rate of reaction rather than completely suppressing it. Cytochrome oxidase activity seemed to be more sensitive to Piromen than succinoxidase activity. Similar studies utilizing mouse monocytes indicated that succinoxidase activity could be reduced or completely abolished, but cytochrome oxidase activity was relatively insensitive to Piromen inhibition. Inhibition of monocyte succinoxidase activity was proportional to concentration of Piromen used.

The excellent technical assistance of Mr. John Meacham is gratefully acknowledged.

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Received June 20, 1960. P.S.E.B.M., 1960, v105.