INHIBITION OF MITOCHONDRIAL RESPIRATION AND PHOSPHORYLATION BY 6-PHOSPHOGLUCONATE

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In a study of the effect of liver supernatant fractions on isolated mitochondria, it was observed that addition of NADP, but not NAD, caused an inhibition of respiration and phosphorylation. The most obvious explanation for this effect was that 6-phosphogluconate, which could have been formed in the system under study, was the inhibitor. The inhibitory activity of exogenous 6-phosphogluconate has been demonstrated and is reported here.

METHODS AND MATERIALS

Mitochondria from normal rat liver, isolated in 0.25 M sucrose by the method of Hogeboom, Schneider and Palade (1948), with the modifications previously described (Devlin and Bedell, 1960), were suspended to a volume of 0.75 to 1.0 g wet weight of liver per ml. Oxygen uptake was measured by conventional manometric techniques, and inorganic orthophosphate by the method of Lowry and Lopez (1946). Hexokinase, crystalline type A, and various salts of 6-phosphogluconate were purchased from Calbiochem. Substrates and other chemicals were from commercial sources and used without further purification.

RESULTS

In manometric experiments where the substrate was added last, 6-phosphogluconate (6-PGA) was found to be an inhibitor of oxygen uptake

of isolated rat liver mitochondria with all substrates tested. As shown in the representative experiments in Table I, 6-PGA at 1.0 mM inhibited both respiration and phosphorylation approximately 40%, with no significant change in the P/O ratio. At 3 mM, respiration and phosphorylation were nearly completely inhibited with DPN-linked substrates but not with succinate. The lack of complete inhibition of succinate oxidation at the higher levels of 6-PGA is similar to the results observed by Lardy, Johnson and McMurray (1958) on the inhibition of respiration by oligomycin, and by Hollunger (1955) for guanidine. Similar inhibitions have been observed in polarographic experiments with ADP as the sole phosphate acceptor, ruling out the possibility that 6-PGA was inhibiting the phosphate "trap" (i.e., hexokinase plus glucose or deoxyglucose).

The inhibitory activity of 6-PGA has been confirmed with a number of commercial samples of different salts, some of which have a purity in excess of 99%. From spectrographic analysis, the samples of

TABLE I

INHIBITION OF RESPIRATION AND PHOSPHORYLATION BY 6-PHOSPHOGLUCONATE

Incubation medium (3.0 ml) contained: 10 mM Tris (hydroxymethyl) aminomethane (pH 7.4), 10.0 mM phosphate buffer (pH 7.4), 6.6 mM $MgCl_2$, 66 mM KCl, 1.0 mM ADP, 16.6 mM glucose or deoxyglucose, 0.01 to 0.02 mg hexokinase (130 to 170 E.U./mg) and rat liver mitochondria (0.8 to 1.5 mgN). Designated substrates, 20 mM, and 6-phosphogluconate at indicated concentrations were added after preincubation (7 minutes). Incubation for 15 minutes at 30°C.

Substrate	6-PGA	$\triangle O_2$	$\triangle P_i$	P/O
	mM	μAtoms	μMoles	
Succinate	-	13.4	25.8	1.9
	1.0	8.3	19.7	2.4
	3.0	5.8	1.8	0.3
β-Hydroxybutyrate	-	6.6	17.8	2.7
	1.0	3.8	9.9	2.6
	3.0	1.2	0.0	0.0
Glutamate + Malate	-	10.0	28.2	2.8
	1.0	5.8	15.6	2.7
	3.0	0.9	0.0	0.0

6-PGA contained only traces of heavy metals which could not have caused the inhibition. In the original study of this effect it was reported (Devlin and Barnes, 1964) that high concentrations of some samples of glucose-6-phosphate and fructose-6-phosphate also had inhibitory activity. Repurification of these samples, however, removed the activity, suggesting the presence of an inhibitor. Studies of the effect of impure samples of glucose-6-phosphate in comparison with 6-PGA suggest that the impurity in the glucose-6-phosphate was different from 6-PGA.

As reported in Table II, addition of 6-PGA to the mitochondria during the preincubation or together with the substrate at zero time caused a marked inhibition, whereas the inhibition was much less if 6-PGA was added five minutes after the addition of substrate, at which time the rate of respiration was maximal. Addition of hexokinase or deoxyglucose, rather than substrate, to initiate respiration also decreased the degree of inhibition by 6-PGA (Table II). These results suggest that the interaction of 6-PGA with its site of inhibition apparently depends on the

TABLE II

EFFECT OF PREINCUBATION MEDIUM AND TIME OF ADDITION OF 6-PHOSPHOGLUCONATE ON THE INHIBITION

Incubation medium as in Table I, except for times of addition as noted. 6-Phosphogluconate (3.0 mM) added during preincubation (-7 minutes), at zero time (0 minutes), or after initiation of active respiration (+5 minutes).

Substrate	Zero Time Addition	Time of Addition of 6-PGA	ΔO_2	ΔP_i
		Minutes	μAtoms	μMoles
Pyruvate	Substrate	None	7.1	22.5
+ Malate	Substrate	-7	1.0	0.0
	Substrate	0	1.3	0.0
	Substrate	+5	5.8	15.5
Glutamate	Substrate	None	10.2	29.9
+ Malate	Substrate	0	1.2	0.0
	Deoxyglucose	None	9.9	29.5
	Deoxyglucose	0	7.9	23.5

state of the mitochondria at the time of addition. The effect of 6-PGA cannot be attributed to a drastic alteration in the structure of the mitochondria, because 6-PGA did not induce a swelling of mitochondria when assayed in a phosphorylating system.

6-PGA also caused an inhibition of the respiration stimulated by 2,4-dinitrophenol when measured either in the presence or absence of a phosphate acceptor system (Table III). These results suggest that in contrast to results with oligomycin (Lardy and McMurray, 1959; Estabrook, 1961) the inhibition by 6-PGA cannot be attributed to an inhibition of the terminal site in oxidative phosphorylation and must either be on the respiratory chain or at a site prior to the point of action of dinitrophenol. This is also supported by the observation (Table IV) that 6-phosphogluconate had no effect on the dinitrophenol-stimulated ATPase. As reported in Table IV, 6-PGA had no effect on either the latent or Mg^{++}-ATPase, which is additional evidence that 6-PGA was not causing a disruption of the mitochondria; aged or disrupted mitochondria have a high level of both latent and Mg^{++}-ATPase.

TABLE III

EFFECT OF 6-PHOSPHOGLUCONATE ON
2,4-DINITROPHENOL STIMULATED RESPIRATION

Incubation medium as in Table I. 20 mM glutamate as substrate; 3.0 mM 6-PGA and 0.05 mM 2,4-dinitrophenol where indicated. Phosphate acceptor - hexokinase plus glucose.

Exp. No.	Omissions and Additions	ΔO_2	ΔP_i
		μAtoms/20'	μMoles/20'
1	Complete	8.6	26.9
	Complete + 6-PGA	1.3	0.0
	Complete + 2,4-dnp	9.8	0.0
	Complete + 2,4-dnp + 6-PGA	0.8	0.0
2	Minus Phosphate acceptor	2.1	2.8
	Minus Phosphate acceptor + 2,4-dnp	9.8	0.0
	Minus Phosphate acceptor + 2,4-dnp + 6-PGA	1.2	0.0

TABLE IV

EFFECT OF 6-PHOSPHOGLUCONATE ON ATPase

Incubation medium: 100 mM KCl, 10 mM Tris (hydroxymethyl) aminomethane (pH 7.4), 5.0 mM ATP, and where indicated 5.0 mM $MgCl_2$, 0.1 mM 2,4-dnp and 3.0 mM 6-PGA. Values are μmoles P_i released/10^7/mgN.

Additions	P_i Released	
	-	+ 6-PGA
-	0.5	0.8
+ $MgCl_2$	0.7	0.8
+ 2,4-dnp	18.4	17.6

A similar lack of alteration of the ATPase activity has been observed if the mitochondria were preincubated with 6-PGA for 5 minutes prior to addition of ATP.

DISCUSSION

The results presented suggest that 6-phosphogluconate could be inhibiting respiration and phosphorylation at a site on the phosphorylating mechanism but not at the terminal transphosphorylation step or at the site where dinitrophenol acts to short circuit the phosphorylating mechanism. This site of action could be similar to that observed for guanidine (Hollunger, 1955; Chance and Hollunger, 1963). Witonsky and Johnson (1964) have proposed a similar site of inhibition for dianemycin. Since 6-phosphogluconate is formed in tissues by the oxidation of glucose-6-phosphate, it is possible that this phosphate could function in a control mechanism whereby carbohydrate metabolism exerts an effect on respiration. The possibility that 6-PGA has a role in the Crabtree effect is now under investigation.

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