AEROBIC GLYCOLYSIS AND MITOCHONDRIAL SWELLING

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Nearly half a century ago Otto Warburg discovered that tumor cells form lactate in aerobiosis whereas normal cells do not. Somehow the respiratory activity of tumor mitochondria seems unable to inhibit the formation of lactate. Warburg claimed that "aerobic glycolysis results if the respiration of growing cells is injured, whether by diminishing its extent or by interfering with the relationship which holds between respiration and glycolysis..... If the respiration of a growing cell is disturbed, as a rule the cell dies. If it does not die, a tumour cell results". (Warburg, 1930).

Criticism of Warburg's ideas arose from two main points: 1) Some normal tissues, for example, brain, kidney medulla, testis, and retina, have aerobic glycolysis (see review by Dickens, 1955). 2) It has not been possible to find any respiratory deficiency in mitochondria isolated from tumor tissues (Weinhouse, 1956). However, even if the ability of tumor mitochondria to oxidize substrates of the Krebs cycle dehydrogenases is normal, alterations in the transport of electrons from the glycolytically generated NADH to the respiratory chain could interfere with the relation which holds between respiration and glycolysis.

Assuming that intact mitochondria are impermeable to external NADH it has been postulated that in most normal cells the transfer of electrons from this cofactor to the respiratory chain is performed by means of different "shuttles". When these shuttles fail to operate, the NADH becomes available to the lactic dehydrogenase and aerobic glycolysis appears (Boxer and Devlin,

1961). However, since the impermeability of mitochondria to external NADH has been recently challenged (Cereijo-Santalò, 1966a), it seemed of interest to study the relation between the capacity of mitochondria to swell and their ability to inhibit the formation of lactate. It was found that mitochondria from tissues which have aerobic glycolysis are resistant to swelling agents and are unable to inhibit lactate formation in vitro, while those from tissues which do not have aerobic glycolysis, swell readily and inhibit strongly the formation of lactate.

Experimental details. Mitochondria were isolated in 0.25 M sucrose following essentially the method of Schneider and Hogeboom (1950). (Brain mitochondria were also prepared in 0.44 M sucrose (Lørtrup and Zelander, 1962)). The mitochondrial pellets obtained were suspended in 0.154 M KCl. The supernatant fraction of a rat brain homogenate centrifuged at 15,000xg for 90 minutes was used as the glycolytic system.

The optical density of mitochondria was measured as previously described (Cereijo-Santalò, 1966b).

The glycolytic medium was similar to that described by Aisenberg et al. (1957): 10 mM glucose, 50 mM tris-histidine (pH 7.4), 5 mM potassium phosphate (pH 7.4), 40 mM nicotinamide, 1 mM ATP, 0.5 mM NAD, 5 mM $MgCl_2$, and KCl to isotonicity. Final volume, 4.0 ml. Supernatant fraction equivalent to 200 mg of rat brain, and mitochondria equivalent to 0.5 gm of tissue (tumor mitochondria equivalent to 2.0 gm of tissue).

Results.

Swelling. Mitochondria were isolated from two groups of tissues: A) Liver and kidney cortex which do not have aerobic glycolysis, and B) brain, testis, kidney medulla, and tumor which do form lactate in aerobiosis. Mitochondria from both groups of tissues showed a slight decrease in optical density when incubated at 24°C in isotonic KCl medium at pH 7.0, which corresponds to the so-called "spontaneous swelling" (Table I, "Control"). This swelling of mito-

chondria was considerably increased when the molarity of the medium was lowered to 63 mM, which indicates that all mitochondria tested behave as good osmometers. However, the swelling induced by the acidity of the medium under isotonic conditions (Cereijo-Santaló, 1966b) was observed only in mitochondria obtained from tissues of group A. Similarly, active swelling induced by succinate oxidation or by the addition of calcium or phosphate (both in the presence of succinate) was observed only with mitochondria from tissues of group A. (In group B only rabbit kidney medulla mitochondria showed some active swelling).

Table I

Variability of mitochondrial response to swelling agents

Mitochondria incubated at 24°C for 30 minutes in 0.154 M KCl plus tris-histidine (25 mM, pH 7.0), unless otherwise indicated. Additions: 7 mM sodium succinate, 5 mM $CaCl_2$, and 5 mM potassium phosphate (pH 7.0) as shown.

	Δ Optical Density					
Mitochondria:	Control	63mM	pH 5.0	Suc.	Suc. Ca^{++}	Suc. Pi
Rat liver	.040	.200	.205	.120	.360	.410
Guinea Pig liver	.020	.275	.130	.070	.245	.170
Guinea Pig kidney cortex	.065	.230	.120	.170	.190	.180
Rabbit liver	.090	.280	.190	.180	.280	.320
Rabbit kidney cortex	.070	.180	.120	.100	.190	.200
Rat brain	.030	.130	.015	.020	.030	.030
Guinea Pig brain	.025	.130	.005	.015	.020	.020
Guinea Pig kidney medulla	.012	.080	.000	.020	.020	.020
Guinea Pig testis	.065	.135	.025	.070	.065	.060
Rabbit brain	.030	.135	.030	.035	.030	.030
Rabbit kidney medulla	.090	.210	.075	.100	.180	.175
Rabbit testis	.070	.160	.010	.070	.065	.070
Human colon carcinoma	.075	.140	.030	.050	.050	.040
Rat carcinoma (Walker 256)	.090	.160	.000	.095	.090	.100

Lactate formation. Table II shows that mitochondria from tissues of group A inhibited the production of lactate by rat brain supernatant fraction, while those from group B did not. Under similar experimental conditions (with the exception that a more dilute suspension of mitochondria was used) it was observed that the optical density of rat brain and tumor mitochondria did not change significantly, while that of rat liver decreased considerably. There-

fore it appeared that swelling and inhibition of lactate formation are parallel processes.

Table II

Change in lactate production of rat brain supernatant fraction induced by mitochondria from different tissues

Rat brain supernatant fraction incubated for 30 minutes at 37°C under the conditions described in Experimental details.

Mitochondria:	Lactate mM	% Change
None	7.0	
Rat liver	2.1	- 70
Guinea Pig liver	3.0	- 57
Guinea Pig kidney cortex	3.5	- 50
Rabbit kidney cortex	2.9	- 59
Rat brain	7.9	+ 13
Guinea Pig kidney medulla	7.0	0
Rabbit kidney medulla	8.5	+ 21
Human colon carcinoma	8.5	+ 21
Rat carcinoma (Walker 256)	8.0	+ 14

Table III

Effect of pH and sucrose on the inhibition of lactate formation by liver mitochondria

Supernatant fraction equivalent to 200 mg of rat brain incubated at 37°C for 30 minutes in the medium described in Experimental details, with buffer at pH indicated. Additions: mitochondria equivalent to 0.5 gm of rat liver, 20 μM cytochrome c, and 0.3 M sucrose as shown. Optical density was measured under the same experimental conditions but with a more dilute suspension of mitochondria.

	Lactate (mM)			
Additions	7.0	7.5	8.0	8.5
None	5.3	6.6	6.6	5.8
Mitochondria	1.0	3.0	3.9	5.0
Mitochondria + cyt. c	0.0	0.6	2.1	2.9
Mitochondria + sucrose		6.7		
Mitochondria + sucrose + cyt. c		2.0		

	Δ Optical Density			
	7.0	7.5	8.0	8.5
Mitochondria	.480	.330	.280	.110
Mitochondria + sucrose		.090		

Table III shows that the inhibition of lactate formation by liver mitochondria was abolished either by increasing the pH of the incubation medium

or by the addition of 0.3 M sucrose, both conditions under which swelling of mitochondria is markedly diminished. (0.3 M sucrose did not modify the rate of lactate formation by the supernatant fraction alone). Thus, under either of these conditions, liver mitochondria behave, in this respect, as tumor mitochondria. This table shows also that the effects of sucrose and alkalinity nearly disappeared in the presence of cytochrome c.

Discussion. It is known that mitochondria from different tissues vary in their capacity to swell. Thus, in contrast to the great extent of swelling observed with liver mitochondria, the mitochondria from brain, testis (Tapley and Cooper, 1956; Spirtes, 1961), and tumor (Emmelot, 1961; Utsumi et al., 1965) are essentially resistant to swelling agents. Similarly, it is known that tumor mitochondria do not inhibit the formation of lactate in vitro (Aisenberg et al., 1957). Therefore, in this respect, our results simply confirm and extend previous findings.

However, our results do suggest a link between the two phenomena of mitochondrial swelling and mitochondrial inhibition of lactate formation. It is possible that mitochondria which swell during glycolysis become permeable to external NADH and, therefore, actively compete with lactic dehydrogenase for this reduced cofactor. (Attempts are now being made to test this view by first finding the conditions, if any, under which tumor mitochondria would swell). The swelling developed during glycolysis could be due to the binding of Mg^{++} by the glycolytically generated ATP (Cereijo-Santalò, 1966a) or to the acidity of the medium. (*)

(*) This statement implies that the acidity induced by glycolysis is not due to the accumulation of "lactic acid". As Colowick (1955) has pointed out, the oxidation of 3-phosphoglyceraldehyde gives rise to an acyl phosphate and the transfer of this acyl group to ADP is accompanied by the absorption of an equivalent acid. Therefore, the "acid" formed in the oxidative reaction of glycolysis will be released if and when the ATP is split. Thus, the glycolytic reaction itself is isohydric:

$$\text{Glucose} + 2\ HPO_4^{2-} + 2\ ADP^{3-} = 2\ \text{Lactate}^- + 2\ ATP^{4-} + 2\ H_2O$$

The reaction which splits the glycolytically generated ATP is responsible for the acidity "induced" by glycolysis.

The observation that sucrose, which inhibits swelling, releases the inhibitory action of liver mitochondria on lactate formation, suggests a cause-effect relationship between the two phenomena. It is true that sucrose inhibits other mitochondrial functions, but since cytochrome c is able to abolish the effect of sucrose it appears that mitochondrial permeability to external NADH is involved. Furthermore, the observation that an increased pH has the same effect as that of sucrose indicates that the swelling is due to the acidity which accompanies glycolysis.

A more detailed report of this investigation will be published elsewhere.

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