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Effect of Iodoacetate and Iodoacetamide on Oxygen Uptake of Heart Mitochondria

Iodoacetate and iodoacetamide have been used as specific inhibitors of the Embden-Meyerhof pathway of glycolysis, the site of inhibition being at the triose-phosphate dehydrogenase. Early reports (*1*) indicated that iodoacetate at low concentrations inhibited anaerobic glycolysis and respiration with glucose but not the oxygen uptake induced by addition of pyruvate or lactate. More recent studies (*2*) have shown that the oxidation of pyruvate may be reasonably sensitive to iodoacetate. A study of the direct effects of iodoacetate and iodoacetamide on the aerobic oxidation of pyruvate and cycle intermediates by mitochondria would provide more information on their effects on respiration and give a basis for the judicious use of a particular concentration of these inhibitors to inhibit specifically the glycolytic pathway.

The preparation of the rat heart mitochondrial suspension and the manometric measurement of oxygen uptake were made according to the methods of Montgomery and Webb (*3*). The results are summarized in Table 1. Both inhibitors at a concentration of 1.0 m*M* produced distinct inhibition with all substrates, the strongest inhibition being observed in the oxidation of pyruvate and α-ketoglutarate, which may indicate the sensitivity of systems involving coenzyme A and lipoic acid. However, the lower concentrations also produced definite inhibitions which cannot be ignored in respiratory studies. It may be noted that iodoacetate was generally more effective than iodoacetamide. In order to produce complete inhibition of triose-phosphate dehydrogenase and glycolysis, concentrations of 0.2 to 0.5 m*M* must be used in most cases, and thus the present results indicate that a complete inhibition of glycolysis is usually accompanied with some effect on respiration (*4*).

Table 1. Effects of iodoacetate and iodoacetamide on the mitochondrial oxidation of various substrates. The reaction medium contained 121 m*M* KCl, 20 m*M* potassium phosphate buffer (*p*H 6.8), 0.01 m*M* cytochrome *c*, 5 m*M* $MgCl_2$, 1m*M* adenosine monophosphate, 0.5 m*M* adenosine triphosphate, and 5 m*M* substrate. The temperature was 37°C. The mitochondrial suspension was incubated for 10 minutes with the inhibitors in the medium, and the oxygen uptake was determined over a period of 1 hour.

Substrate	Change (%) at various concentrations		
	0.01 m*M*	0.10 m*M*	1.0 m*M*
Iodoacetate			
α-Ketoglutarate	− 6.3	−33.3	−75.6
Malate	− 7.4	−20.0	−63.9
Pyruvate + malate	− 4.3	−43.0	−85.6
Succinate	− 3.8	− 8.0	−61.2
Citrate	+ 4.6	− 8.1	−34.6
Isocitrate	+15.0	−15.3	−35.0
Iodoacetamide			
α-Ketoglutarate	− 6.4	−17.2	−76.3
Malate	− 1.0	−21.0	−35.3
Pyruvate + malate	− 9.4	−12.6	−79.7
Succinate	− 2.5	−17.0	−43.1
Citrate	−16.1	−14.9	−44.1
Isocitrate	−12.0	− 7.3	−29.1

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Carbon-14 Activity of Some Heat-Degradation Products of Milk Containing Lactose-1-C^{14}

The course of heat-induced lactose-protein interaction in milk has been followed with the aid of lactose-1-C^{14} (*1*). Use of labeled lactose also appeared attractive for investigation of the sugar's decomposition under these conditions. Of the many fragments known to be formed (*2*), formic acid, furfuryl alcohol, and maltol (3-hydroxy-2-methyl-pyrone-4) were evaluated in these experiments. It has been proposed that formic acid is derived from carbon atom No. 1 and furfuryl alcohol from carbon atoms 2 through 6 in the glucose moiety of lactose (*3*). Maltol results rather uniquely from the heat-induced interaction of reducing disaccharides with amino compounds (*2*). It has been detected in evaporated milk, baked cereals, bread crust, and roasted malt, among other places (*4*).

Table 1. Levels of C^{14} activity found in some heat-degradation products of skim milk containing lactose-1-C^{14}.

Compound	Carbon (atom/mole)	Activity of $BaCo_3$ (Count/min mg)		Lactose/product C^{14} ratio
		Found	Theory*	
Lactose	12	8.7		
Formic acid	1	53	104	1/0.51
Maltol	6	14	17.4	1/0.81
Furfuryl alcohol	5	0.8	20.9	1/0.04
Naphthyl urethane	16	0.0	6.5	
3,5-Dinitrobenzoate	12	0.0	8.7	

* Based on molar transfer of 1 atom of C^{14}.

The three compounds in question were recovered and purified from heated (121°C for 4 hours) condensed skim milk (30 percent total solids) to which lactose-1-C^{14} (National Bureau of Standards) had been added. Steam distillation was used to isolate the compounds from the heated milk. Maltol and furfuryl alcohol were recovered from this distillate by ethyl ether extraction and were purified as described elsewhere (*3, 5*). Formic acid was recovered by neutralizing a portion of the distillate to *p*H 7.5 and evaporating the solution to dryness under vacuum (*6*). The crude formate was selectively converted to CO_2 by the method of Osburn *et al.* (*7*). This CO_2, samples of furfuryl alcohol and its derivatives, maltol and lactose, the latter from the unheated product, were converted to $BaCO_3$ (*8*). Radioactivity in these preparations was determined with a windowless flow gas Geiger-Müller counter and decade scaling unit.

The data thus secured (Table 1) reveal that carbon atom No. 1 of lactose is involved in the formic acid and maltol, but not in the furfuryl alcohol. A preliminary experiment yielded essentially the same findings with the exception that some activity was detected in the furfuryl alcohol (*9*). Further investigation of the alcohol and two carefully authenticated derivatives of it, as shown in Table 1, revealed that it had no activity.

Under the rigorous heating conditions employed in these experiments, a number of carbon sources could contribute to formate; however, carbon 1 of lactose