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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

appears to be the principal origin. Theories which propose that the carbon skeletons of furfuryl alcohol and maltol of heated milk derive from carbon atoms 2 to 6 and 1 to 6, respectively, in the glucose moiety of lactose remain attractive in light of these findings (*10*).

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Gamma Rays from Local Radioactive Sources

There is considerable interest at the present time concerning the possible effects of man-made radiations on man himself. Because one source of these radiations is of world-wide extent, the interest has also become world-wide. Although considerable literature now exists on the subject of man-made radioactive contamination, on the one hand, and on the biological effects of radiation, on the other, the actual importance of the first as far as the second is concerned has often been obscure. It is thought desirable at this time to present some independent experimental data that will allow individuals to reach their own conclusions.

As early as 1928, R. A. Millikan became interested in the gamma rays emitted by local radioactive materials in the soil and rock at various localities in order to determine the effect of these radiations on the cosmic-ray measurements in which he was primarily interested. These measurements extended from California into the Rocky Mountain area and on up to Churchill, Manitoba (*1*). They probably represent a unique series of measurements, since they were made before man-made contamination became widespread.

An ionization chamber measures directly the quantity of interest as far as the biological effects of gamma rays are concerned, and this is the instrument here employed. One of the instruments Millikan made and calibrated is still in good condition after 26 years and is very convenient to use. A recent redetermination of the absolute value of the calibration (*2*) agrees with Millikan's value to 0.3 percent. In this survey, Millikan's instrument has been used for some of the measurements, and a more modern ionization chamber (*3*) for others. The two give essentially the same answer. Both were used unshielded in the measurements reported here.

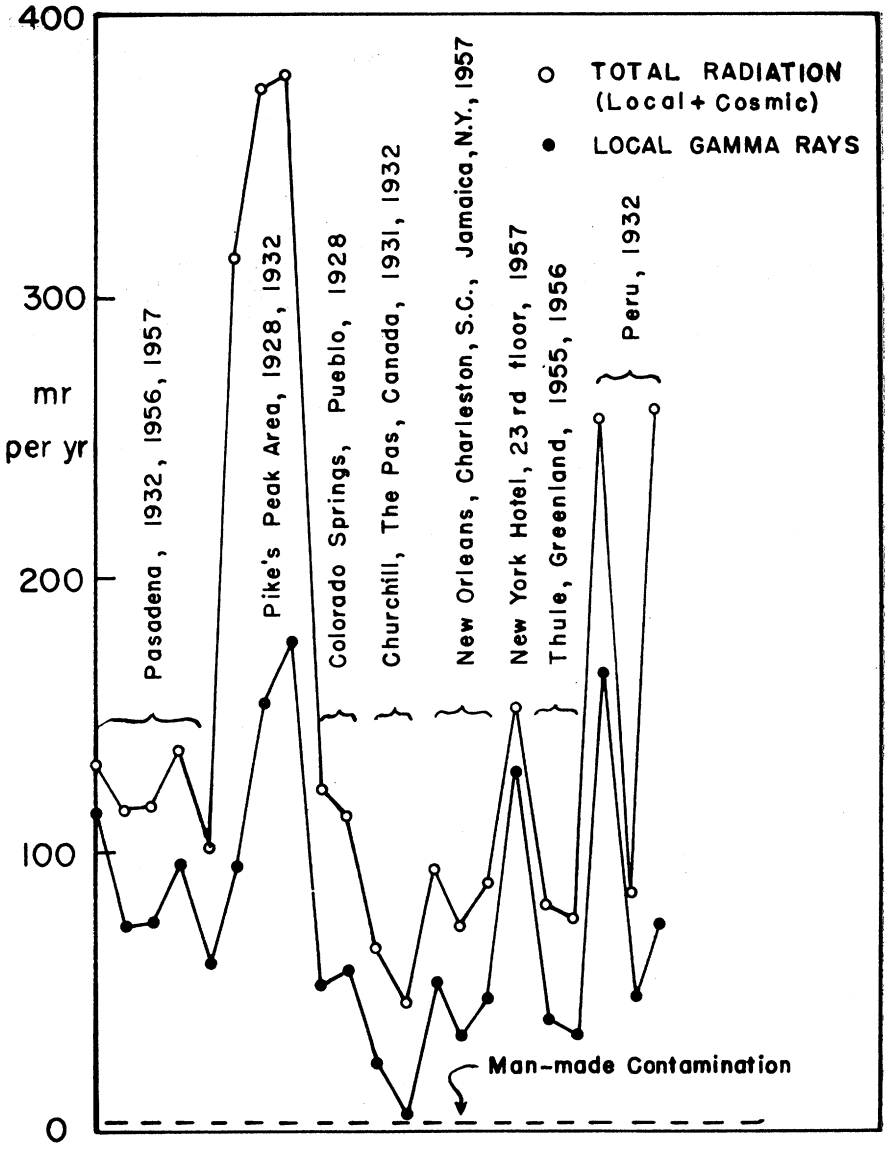

Fig. 1. "Noise level" of gamma rays and cosmic rays in the Western Hemisphere. Abscissas roughly increase with increase of distance from Pasadena. The amount of man-made contamination is taken from the National Academy of Sciences report, *Biological Effects of Atomic Radiation* (*7*). As is stated in that report, ". . . U.S. residents have, on the average, been receiving from fall-out over the past five years a dose which, if weapons testing were continued at the same rate, is estimated to produce a total 30-year dose of about 0.1 roentgen" (an average of 3 mr per year).

In Fig. 1, most of the values taken during the years have been entered. The ordinates are in milliroentgens (mr) per year. To convert into ion pairs per cubic centimeter, per second in 1 atmosphere of air, divide the ordinates by 15. The various stations are plotted as abscissas with the same increment from one to the other. Roughly, the stations get farther from Pasadena with increase in abscissa. The chief reason for plotting in this manner was to bring out the variability of radioactivity from one station and region to another.

Measurements were made of the total radiation at a given station; then the known contribution from cosmic rays (*4*) was subtracted to get the effect of the gamma rays from local radiation only.

In the Rocky Mountain region, the local radiation is high, presumably because of the granite, which is known to