Cardiac Metabolic Response to Hyperbaric Oxygen[1] (34637)

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Elevated pressures of oxygen have produced myocardial changes ranging from increased synthesis of protein (1) to diminished cardiac output, decreased myocardial blood flow, and lowered oxygen, lactate, and pyruvate uptake (2). The present experiments were designed to determine if alterations in cardiac aerobic and anaerobic pathways are produced when rats are subjected to hyperbaric pressures of oxygen for periods of time sufficient to produce evidence of central nervous system toxicity.

Methods. Nonfasted, male albino rats of the Buffalo strain (303 – 489 g), fed Purina lab chow, were placed in a hyperbaric chamber. The chamber was thoroughly flushed with oxygen, and the animals were subjected to 45 pounds per square inch gauge pressure (psig) with 100% oxygen at a rate of 5 psig/min. The animals were exposed to this pressure for a period of approximately 3 hr; at the end of this time, the animals exhibited central nervous system manifestations of oxygen toxicity (hyperexcitability and, frequently, convulsions). After slow decompression (3 psig/min) the animals were removed from the chamber and decapitated. The hearts were rapidly excised from the rats and, for the lactic acid generation and oxygen consumption studies, the left ventricle and interventricular septum were isolated and homogenized in 0.25 *M* sucrose. The procedures for both the lactic acid production and oxygen consumption studies were essentially as those reported in a previous experiment (3). The supernatant from the homogenate was divided into two portions; one was placed in Warburg flasks for oxygen consumption determinations, and the other portion was incubated in an atmosphere of 95% nitrogen–5% carbon dioxide for lactic acid determinations. Left ventricular and interventricular tissue was used for these studies, because this tissue preparation was found to be more stable for oxygen consumption studies than the right ventricular homogenate (unpublished data). The lactic acid determinations were made by a modification of the Hohorst method which requires the generation of reduced nicotinamide-adenine dinucleotide (NADH) (4). The optical density of NADH was then read in a Gilford spectrophotometer at 340 mμ. The enzymes for both the lactic acid and adenosine-5′-triphosphoric acid (ATP) procedures were obtained from the Calbiochem Company. The ATP levels were determined, with minor modifications, by the method of Lamprecht and Trautschold (5). For determination of ATP concentrations, the hearts were removed rapidly from the rats and plunged into liquid nitrogen. The ventricles were separated from the atria and trimmed while in the frozen state. The frozen ventricular tissue was then ground in a mortar and pestle with 3.5% perchloric acid (14.25 ml of acid/g of tissue). The powdered, acidified tissue was homogenized while in an ice bath, and the homogenate was then centrifuged. The supernatant was brought to a pH of 7.5 with 5 *M* potassium carbonate. Aliquots of the supernatant were analyzed for ATP levels by the enzymatic reduction of nicotinamide-adenine dinucleotide phosphate. The optical density of the reduced nicotinamide-adenine dinucleotide phosphate was determined at 340 mμ in the Gilford spectrophotometer. Control ani-

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TABLE I. Effects of 45 psig of Oxygen on Cardiac Metabolism.[a]

	O_2 consumption[b] (μl/100 mg/hr)	Lactic acid production (μmoles/g)	ATP conc (μmoles/g)
Experimental	374.4 ± 16.0 (11)[c]	863 ± 24[d] (12)	3.84 ± 0.19 (10)
Control	361.5 ± 12.5 (8)	708 ± 20[d] (10)	3.85 ± 0.32 (11)

[a] Based on wet tissue weight; results expressed as means ± SE.
[b] Oxygen consumption for the first hour.
[c] Number of animals per analysis is given in parentheses.
[d] $p < 0.001$.

mals were chosen from the same population and were sacrificed in the rested condition.

The data were analyzed by using the Student's *t* test of significance.

Results. The data which describe the aerobic and anaerobic activity of the heart tissue from animals exposed to the 45 psig of oxygen are presented in Table I. No significant differences were noted in either the ATP concentrations or the oxygen consumption values obtained during the first hour of incubation. The Warburg study was carried on for a 2-hr duration, but the experimental and control values for the second hour were essentially the same (244.9 and 244.3 μl/100 mg of wet tissue wt for experimental and control groups, respectively). The lactic acid production for the experimental group was significantly greater than that found in the controls ($p < 0.001$), and there did not appear to be lactic acid accumulation in the hearts of experimental rats analyzed immediately after the hyperbaric exposure.

Discussion. The toxic effects of oxygen on cardiac tissue homogenates were noted some time ago in the *in vitro* preparation (6), and the difficulties in translating these results to the *in vivo* situation are well known (7). However, there appears to be no evidence of toxicity in cardiac tissue of the exposed animals in the present study. The reduction of ATP in other organs has been cited as evidence of oxygen toxicity by Sanders *et al.* (8). These authors subjected their animals to hyperbaric pressures of oxygen until the rats were convulsing and gasping. The animals in our study were in a similar moribund condition, but the ATP levels were maintained in the cardiac tissue. In addition, the toxic effects of oxygen did not manifest themselves in the oxidative metabolic reactions, as evidenced by the undiminished oxygen consumption values of the experimental animals. An interesting finding is the increased ability of the cardiac tissue of exposed animals to produce lactic acid. This enhanced glycolytic activity, as the result of increased pressures of oxygen, would not have been predicted from the work by Weglicki *et al.* (9). These authors concluded that there was an apparent inability of anesthetized dogs to produce lactate when artificially exercised in elevated pressures of oxygen. In later work Weglicki and colleagues (2) noted that there was no impairment of myocardial substrate utilization for lactate, pyruvate, free fatty acid, and glucose when dogs were exposed to 3 atm of oxygen.

The mechanisms involved in the increased ability of cardiac tissue to produce lactic acid cannot be explained at the present time. The elevated lactic acid production may be the result of release of suppression of the glycolytic system that possibly occurred when the animal was removed from the hyperbaric chamber (the removal of the Pasteur effect). This increased glycolytic activity may be the reverse of that type of phenomenon seen in the hearts of rats forced to deplete their cardiac glycogen by swimming exercise (10). After recovery from exercise the glycogen concentration of the heart remains significantly elevated for a time. This same finding has been reported in rats subjected to acute anoxia (11).

Summary. Rats were exposed to 45 psig of oxygen until central nervous system toxicity was evident (approximately 3 hr). Oxygen consumption, lactic acid production, and ATP concentration were studied in the heart

tissue of these rats after their hyperbaric oxygen experience. Of these 3 parameters only the lactic acid production proved to be significantly different in the experimental animal. Heart homogenates from the exposed rats produced a greater amount of lactic acid than control rats.

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