# Effects of Hyperbaric Oxygenation on Metabolism I. ATP Concentration in Rat Brain, Liver and Kidney.* (30689)

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In 1878, Paul Bert(1) first described the toxic effects of high pressure oxygen on mammalian tissues and suggested that certain metabolic processes such as catabolism of sugars in the blood and oxygen consumption of cells seemed to be impaired. Since 1878, numerous articles have appeared which examined the mechanism by which high pressure oxygen exerts its toxic effects on glucose metabolism in tissue preparations(2-5). Kaplan and Stein(6) demonstrated that when brain tissue slices were exposed to high pressure oxygen there was a decrease in potassium content, an increase in sodium content, and a decrease in intracellular accumulation of glutamate. The same authors(6) suggested that high pressure oxygen exerts its toxic effect on tissues "by reducing the energy available for the establishment of chemical gradients across cell membranes of substances essential for the normal functioning of those cells."

These papers suggest that the toxic effects of oxygen at high pressure *in vivo* could result in a reduction in tissue high energy phosphate compounds, *i.e.*, adenosine-5'-triphosphate (ATP). This study was undertaken to determine if high oxygen pressure affects ATP concentrations in the cerebral hemispheres, the liver and the kidney of the rat.

*Methods.* Male Sprague-Dawley rats weighing between 150 and 225 g were used. All animals were fasted from 18 to 24 hours preceding the experiment. The control group consisted of animals exposed to air at 1 atmosphere. Other groups of animals were exposed to 100% oxygen at 1 and 3 atmospheres for 2 hours, and at 5 atmospheres for 1½ hours. The Bethlehem Steel Corp. table top hyperbaric chamber was used to maintain the desired pressures.

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Since it has been observed that the ATP concentration drops rapidly in anoxic tissues (7), it was essential that special precautions be used in preparation of tissues for ATP analysis. As soon as the animal was taken from the chamber it was anesthetized with ether. The blood supply to the organ remained intact until the organ was removed. After the liver and kidney were exposed the animal was held over the liquid propane container, the organ excised with a single scissors snip, and dropped instantaneously into the liquid propane (—190°C). For brain analyses, the head was positioned in the guillotine so that when one stroke of the blades passed through the cerebellum, the anterior portion of the head fell directly into the liquid propane. In most instances, the head split along the dorsal midline exposing the medial surfaces of the brain, thus expediting the freezing process.

The frozen cerebral hemisphere was rapidly removed from the skull with instruments previously surrounded by dry ice. The frozen brain, liver or kidney was then pulverized, placed in a tared, iced homogenizer, weighed and homogenized in 10% TCA (9 ml of TCA per gram of tissue). The homogenate was added to approximately 400 ml of ice cold distilled water, neutralized with 1 N KOH to pH 6-6.5, and diluted to a final volume of 2 liters for the cerebral hemisphere, and 1 liter for liver and kidney. The ATP concentration of the tissue preparations was measured by the firefly luminescence method (8-11) utilizing diluted firefly extract (Sigma FLE 50) and a Beckman D.D. spectrophotometer(7).

*Results.* The average tissue ATP concentrations, standard deviations, percentages of control values, and the probabilities of being the same as the normals are shown in Table I.

Liver and kidney ATP levels were essentially the same as normal at 1 atmosphere.

TABLE I. Tissue ATP Concentration with High Pressure Oxygen.

| Tissue | Micromoles ATP per gram wet wt* | | | |
|---|---|---|---|---|
| | Normal air | 1 atmosphere 2 hours | 3 atmospheres 2 hours | 5 atmospheres 1½ hours |
| Brain | 3.66 ± .22 ( 8) | 1.57 ± .50 (9) | 2.42 ± .45 (16) | 1.71 ± .39 (8) |
| % | 100 | 43 | 66 | 47 |
| P | | P <.01 | P <.01 | P <.01 |
| Liver | 1.74 ± .36 ( 8) | 1.88 ± .46 (7) | 2.41 ± .60 ( 7) | .97 ± .24 (5) |
| % | 100 | 108 | 139 | 56 |
| P | | P >.5 | P <.01 | P <.01 |
| Kidney | 1.13 ± .16 (12) | 1.05 ± .22 (8) | 1.34 ± .29 ( 7) | .42 ± .09 (5) |
| % | 100 | 93 | 118 | 37 |
| P | | P >.5 | P =.02 | P <.01 |

* Mean ± standard deviation.

At 3 atmospheres liver and kidney showed an increased ATP concentration of 39% and 18% respectively. At 5 atmospheres liver dropped to 56% and kidney dropped to 37% of the control values. The cerebral hemisphere had a 57% reduction in ATP concentration at 1 atmosphere, a 34% reduction at 3 atmospheres, and a 53% reduction at 5 atmospheres.

The condition of the animal varied at time of removal from the chamber. At 5 atmospheres for 1½ hours, the animal was convulsing, frothing at the mouth, and gasping deeply. In contrast, the animal after being subjected to 100% oxygen at 1 and 3 atmospheres for 2 hours, appeared to be mentally alert.

*Discussion.* The present study supports the proposal of Kaplan and Stein(6) that high pressure oxygen exerts its toxic effect upon tissues by reducing available energy. This energy is necessary to establish a chemical concentration gradient across the membrane in order to maintain an ionic equilibrium.

Samson *et al*(12) have hypothesized that the "critical level of viability" for rat brain at 37°C is 1 micromole of ATP/gram of tissue. Sanders(13), however, has shown that in hypoxic rats (5% oxygen) a depression in the ATP level of brain and liver is associated with a significant reduction in the incorporation of amino acids into protein, and in liver, in tissue uptake of amino acids.

The reduction in tissue ATP concentrations found in brain at 1, 3 and 5 atmospheres and in liver and kidney at 5 atmospheres exceeds the fall in ATP concentration found in these tissues in hypoxia (5% $O_2$). Thus it is logical to assume that significant alterations in cellular functions would occur as a result of the low ATP concentrations found in these tissues after high pressure oxygenation. Such alterations in cell functions could, if prolonged, result in irreversible damage to the cell.

*Summary.* 1) Tissue ATP concentrations were determined in the cerebral hemispheres, liver and kidney in rats exposed to air at 1 atmosphere, and to 100% oxygen for 2 hours at 1 and 3 atmospheres, and 1½ hours at 5 atmospheres. 2 Liver and kidney had normal ATP levels at 1 atmosphere and increased ATP levels at 3 atmospheres (39% and 18% respectively). 3 Cerebral hemispheres had markedly depressed ATP concentrations at 1 atmosphere (57%) and 3 atmospheres (34%). 4 Significant decreases in ATP levels occurred in cerebral hemisphere (54%), liver (44%), and kidney (63%) of rats exposed to 100% oxygen for 1½ hours at 5 atmospheres. 5 The significance of the findings is discussed.

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## Effects of Hyperbaric Oxygenation Metabolism II. Oxidative Phosphorylation in Rat Brain, Liver and Kidney.* (30690)

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The concentration of adenosine-triphosphate (ATP) has been shown to drop significantly in rat brain, liver and kidney when the animal developed severe symptoms of oxygen toxicity after exposure to 5 atmospheres $O_2$ for 1½ hours(1). The cause for this reduction in ATP concentration is not known. One possibility is oxygen poisoning of respiration and oxidative phosphorylation enzymes.

Dickens(2) has reported that respiration of brain slices is slowly and irreversibly poisoned by exposure to hyperbaric oxygen. Brain tissue respiration was reduced 25% by 2.9 atmospheres after 117 minutes, and 25%, 50% and 75% after 56, 80 and 105 minutes exposure to 5.08 atmospheres $O_2$. Dickens(3) observed that succinic dehydrogenase activity or rat brain homogenates was irreversibly poisoned by hyperbaric oxygen. Thomas *et al* (4) found a rapid depression of alpha-ketoglutarate dehydrogenase activity in rat brain mitochondria during exposure to 5 atmospheres of $O_2$.

This work was initiated to determine if there is a correlation between the previously observed reduction in tissue ATP concentration in animals exhibiting acute symptoms of oxygen toxicity (5 atmospheres $O_2$, 1½ hours)(1), and irreversible oxygen poisoning of respiration and oxidative phosphorylation processes.

*Methods.* Male Sprague-Dawley rats (160 to 225 g) were used throughout the study. All animals were fasted 18 to 24 hours preceding the experiment with water *ad libitum*. Control studies were obtained from animals exposed to air at 1 atmosphere. Other groups of animals were exposed to 100% $O_2$ for 2 hours at 1 and 3 atmospheres, and for 1.5 hours at 5 atmospheres.

When the animals were removed from the hyperbaric chamber, the tissues were rapidly removed from the animal and homogenized, as previously described(5), at normal air oxygen pressure. The polarographic method of Chance and Williams(6) as modified by Ziegler *et al*(7) and Sanders *et al*(5) was used to determine respiration and oxidative phosphorylation of homogenates at 25°C of the cerebral hemisphere, the liver and the kidney cortex of the rat, with succinate and alpha-ketoglutarate as substrates. The method of Cooperstein, Lazarow and Kurfess(8) as modified by Hall(9) was used to determine the succinic dehydrogenase activity of similarly prepared homogenates.

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