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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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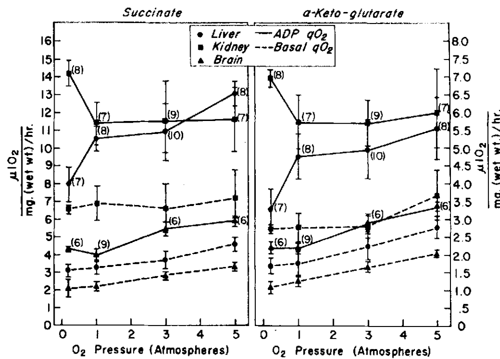

FIG. 1. Hyperbaric oxygen effects on $ADPqO_2$ and basal qO_2.

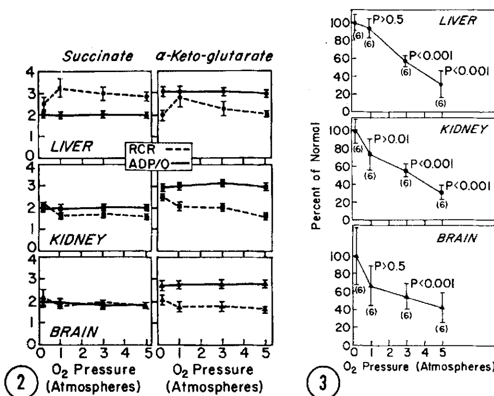

FIG. 2. Hyperbaric oxygen effects on ADP/O and respiratory control ratios.

FIG. 3. Hyperbaric oxygen effects on succinic dehydrogenase activity.

Results. The adenosine-diphosphate-oxygen ratio (ADP/O), the ADP stimulated respiration rate ($ADPqO_2$), the basal respiration rate (Basal qO_2), the respiratory control ratio (RCR) are shown in Fig. 1 and 2 for the 3 tissues for succinate and for alpha-ketoglutarate. Fig. 3 shows the variation in succinic dehydrogenase activity (% of controls) as a function of oxygen pressure in atmospheres.

When returned to normal oxygen tension, all 3 tissue homogenates maintained their efficiency for oxidative phosphorylation, as indicated by the ADP/O ratio, with both substrates. The ATP production capacity, as shown by the $ADPqO_2$, increased with increasing oxygen pressure for the cerebral hemisphere (up 35% and 54% for succinate and alpha-ketoglutarate respectively at 5 atmospheres). Kidney cortex had a 20% reduction in ATP production capacity for both substrates at all elevated oxygen pressures. The reserve ATP production capacity as reflected in the respiratory control ratio (RCR) increased in liver and decreased in the cerebral hemisphere and kidney with hyperbaric oxygen.

Succinic dehydrogenase activity decreased in all 3 tissues with increasing pressure. It should be noted that even though succinic dehydrogenase activity was reduced 69% for liver and kidney cortex, and 57% for brain, from animals exposed to 5 atmospheres O_2, the maximum respiration of tissues from animals exposed to 5 atmospheres O_2 was increased 64% for liver, 35% for cerebral hemisphere, and was reduced only 20% for kidney cortex.

Discussion. Stadie *et al*(10) have observed that rat kidney slices (incubated in a glucose medium), previously exposed to high oxygen pressure (7 atmospheres O_2) showed a 30%, 50%, and 65% reduction from normal oxygen consumption after 1, 2, and 3 hours exposure respectively. However, the same investigators(11) observed that brain slices taken from rats killed by high oxygen pressure showed no significant change in oxygen consumption from normals. Similar preparations of brain slices and homogenates from rats exposed to hyperbaric oxygen, subsequently showed a steadily decreasing rate of oxygen uptake in proportion to the O_2 pressure and exposure time with glucose, fructose, pyruvate or lactate as substrate.

Our results show that the cerebral hemisphere and liver from animals exposed to 5 atmospheres O_2 for 1½ hours, had no decrease but rather an increase, in respiration and oxidative phosphorylation with succinate and alpha-ketoglutarate as substrates. Kidney showed a reduction of 20% in the respiration and oxidative phosphorylation for similar conditions. These data indicate that any inhibition which might occur at these sites of substrate oxidation, as implied by the work of Dickens(3) and Thomas *et al*(4), is reversible when the tissue is returned to normal oxygen tension. Yet our data on succinic dehydrogenase activity of tissues removed from animals exposed to 1, 3, and 5

atmospheres O_2 shows an irreversible reduction of succinic dehydrogenase activity. Liver and cerebral hemisphere homogenates showed increased respiration with succinate, with full efficiency for oxidative phosphorylation. When marked reduction in succinic dehydrogenase has occurred, kidney cortex homogenates showed only slight reduction in respiration with succinate even though succinic dehydrogenase showed a 69% reduction. Thus it is concluded that there is an excess of succinic dehydrogenase within these tissues.

There is no obvious correlation between decreased ATP concentration in the 3 tissues and any irreversible change in respiration and oxidative phosphorylation with succinate and alpha-ketoglutarate. There appears to be a rough correlation between the decrease in ATP concentration after 5 atmospheres O_2, 1½ hours exposure and the reduction in succinic dehydrogenase activity. There was no such correlation seen when the animal was exposed to 1 or 3 atmospheres O_2 for 2 hours.

If there is irreversible damage to tissue respiration as reported by Stadie *et al*(10,11), Dickens(2), then it must occur at some site between glucose and alpha-ketoglutarate. Such irreversible damage was not seen in the respiration of either alpha-ketoglutarate or succinate. The data on succinic dehydrogenase activity are in agreement with Dickens' (3) observation that the activity of succinic dehydrogenase is irreversibly poisoned by hyperbaric oxygen.

Summary. 1 Respiration and oxidative phosphorylation (using succinate and alpha-ketoglutarate as substrates) and succinic dehydrogenase activities were determined in homogenates of the cerebral hemisphere, liver and kidney cortex from rats previously exposed to normal air, and to 100% O_2 for 2 hours at 1 and 3 atmospheres and for 1.5 hours at 5 atmospheres. 2 Increased respiration with both substrates was observed with increased exposure pressure in liver and cerebral hemisphere homogenates while efficiency of oxidative phosphorylation was retained. 3 Kidney homogenates showed decreased respiration with both substrates with normal efficiency for oxidative phosphorylation at all elevated oxygen pressure exposures. 4 Succinic dehydrogenase activity decreased in all 3 tissues with increasing pressure of O_2 exposure. 5 Succinic dehydrogenase concentration is in excess of the amount required to maintain maximum respiration in all 3 tissues with succinate as the substrate. 6 Any inhibition of respiration and oxidative phosphorylation of succinate and alpha ketoglutarate in the cerebral hemisphere, liver and kidney cortex by hyperbaric oxygen exposures of rats, is reversible on returning tissues to normal oxygen tension. 7 There is no correlation between previously observed depressions in rat cerebral hemisphere, liver and kidney cortex ATP concentration with hyperbaric oxygen(1), and irreversible changes in respiration and oxidative phosphorylation of succinate and alpha-ketoglutarate. 8 There appears to be a correlation between the depression of ATP concentration of the cerebral hemisphere liver and kidney and the reduction in succinic dehydrogenase activity at exposure levels (5 atmospheres O_2, 1.5 hours) which produce acute symptoms of oxygen toxicity in the rat. No such correlation is seen at 1 and 3 atmospheres O_2 exposure for 2 hours.

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