

## THE IMPACT OF INTERRUPTIONS IN HYPERBARIC OXYGEN EXPOSURES ON THE DEVELOPMENT OF OXYGEN TOXICITY IN RATS

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### ABSTRACT

The toxic effect of oxygen in hyperbaric conditions requires us to seek measures that would prevent or delay its development. One of such measures consists in the application of interrupted exposure during which the oxygen will be replaced for a short period of time with air. For the purpose of explaining the efficiency of such a procedure, tests were conducted on 26 rats, which were subjected to uninterrupted oxygen exposure at the pressure of 4 ata for the period of 4 hours (control group) or interrupted exposure according to the scheme: 30 minutes of oxygen – 15 minutes of air within the time of 5 hours and 45 minutes. The research was carried out in an experimental hyperbaric chamber enabling constant observation of the animals, with the composition of the breathing gas analysed by means of chromatographic analysis. In the control group the initial symptoms of oxygen toxicity were noted after 2 hours, and after 4 hours 100% of the animals were affected. Post-mortem examination revealed vast morphological lesions in the lungs. In the group subject to interrupted exposure the first toxicity symptoms were observed after 3 hours, whereas after 4 hours they were noted only in 20% of the animals and the morphological changes found in the lungs were minimal.

An increase in the animals' tolerance to the effect of oxygen was dependent on the type of scheme applied with regards to the interruptions in oxygen exposure at a given pressure (thus lowering its toxic effect on the system (brain or lungs)). The protective mechanism of such interruptions has not been thoroughly explained and as such is being discussed here in this work. An important role may be played by periodic filling of pulmonary alveoli with nitrogen bringing back their ventilatory function or a break in the effect of oxygen, which has an adverse impact on proper surface tension of pulmonary alveoli. +/- "The impact of interrupted exposures during hyperbaric oxygenation on rat mortality".

Key words: interrupted exposures, experimental studies, animal model, hyperbaric oxygenation.

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## INTRODUCTION

During different types of activity, such as diving or industrial activities but also in the treatment of various medical conditions, humans often encountered oxygen concentrations exceeding those present in the ambient atmosphere.

These concentrations have a toxic effect even though there is no applied overpressure. This effect becomes stronger with an increase of the pressure surrounding the system, when the percentage content of oxygen in a breathing mix is subject to a significant elevation, i.e. during hyperbaric oxygenation.

Such conditions are present, among other things, in:

- medicinal hyperbaric chambers used in hospitals in routine treatment of CO poisoning, anaerobic bacterial infections, as well as different indications.
- diving with the use of helium-oxygen mixes.
- diving with closed-circuit (oxygen) or semi-closed circuit apparatuses (nitrogen-oxygen mixes).
- oxygen decompression.
- medicinal decompression using, according to some tables, partial breathing with oxygen already at 2.8 ata, i.e. at the 18-metre station.

The dynamic development of the diving technique aimed at a fast conquest of the continental shelf requires us to pay close attention to the primary ingredient of each breathing mix, i.e. the oxygen.

The toxic effect of oxygen in hyperbaric conditions has been known for a long time, however, we still lack certainty as to the exact safety thresholds, both in relation to the lungs and the central nervous system.

Not that long ago, the norms defining the range of pO<sub>2</sub> between 150-450 mm Hg [6,12] were established, as being safer for longer exposures, in particular with regard to the lungs. Some researchers claim that there is no defined threshold for oxygen toxicity and each increase beyond its regular content in the atmospheric air constitutes a potential hazard [9].

Pharmacological prophylaxis methods used in relation to oxygen toxicity, despite appearing not to be too invasive, proved deceptive and dangerous. It is quite easy to cure the symptoms of acute oxygen toxicity in the form of spasms with medicines inducing nervomuscular block (curari) or to delay lung damage by administering adrenaline compounds (e.g. largactil) or anaesthetics (e.g. pentobarbital), thus eliminating the nerve component in the oxygen-induced lung injury.

However, actual prevention against the toxic effect of oxygen aims at inhibiting the development of the process occurring in the central nervous system, whose only symptom, and usually an early one, consists of convulsions. And so far the administration of a variety of chemical compounds – at least in doses not causing serious side-effects – fails to produce the desired effect.

The only effective method for preventing the toxic effects of oxygen on the system is avoidance of oxygen exposures, otherwise no other method allows to completely protect humans or other mammals against oxygen toxicity during exposures to such oxygen partial pressures that may be toxic depending on the time elapsed.

One of the methods that may extend the so-called safe latency period, and at the same time increase the system's tolerance to oxygen activity, involves an interruption in the exposure to high oxygen pressure (concentration) by implementing a period of breathing with air or a breathing mix with low oxygen content or approximated to regular oxygen content in the atmospheric air. This method was applied for humans and animals with the use of different experimental schemes, both with atmospheric [3,13] and hyperbaric pressures [6,10,11].

The said tests proved that the time elapsed until the development of oxygen toxicity symptoms, or survival time, may be significantly prolonged with proper adjustment of the scheme: oxygen exposure – interruption.

The scheme will be probably different with regard to the pulmonary and cerebral form of oxygen toxicity and optimal variations will require separate determination for different proportions between "oxygen concentration – time". In some countries the principle of interrupted exposures is implemented as a day-to-day diving practice.

In England all oxygen dives to depths greater than 7.5 m are periodically interrupted by returning to the depth of 3 metres or even to the surface. However, we lack accurate information whether it is possible to extend the safe exposure period for already critical values of 4 ata.

By continuing our works on the issue of hyperbaric oxygenation [2,7] it was our objective to examine how a selected scheme influences the development of oxygen toxicity in experimental animals – rats.

## MATERIAL AND METHODS

The test was carried out on 26 Wistar rats, males, with the body weight of ca. 200 g, remaining on a standardised diet. The control group consisted of 16 animals, whereas the test group of 10.

The research was conducted in an experimental hyperbaric chamber for animals with the capacity of ca. 30 l, which was filled with oxygen or air according to the following scheme: 30 minutes of oxygen exposure – 15 minutes of air exposure (fig. 1) until the total time of 4 hours in the control group (constant oxygen exposure) and 5 hours 45 minutes in the group with interrupted exposures.

During the entire time of the experiment the pressure in the chamber was maintained at 4 ata; the replacement of breathing gases was performed without pressure changes and commonly lasted about 2 minutes.

After each change of the breathing gas the chamber's composition was analysed with the use of gas chromatography [1] with hydrogen as the carrier gas.

The content of oxygen in the period of oxygen exposure was equal to 90% ± 3%, whereas during air exposure 22% ± 2%. The remaining gas was mainly nitrogen. Carbon dioxide content was not measured, however the applied gas flow in the chamber, amounting to ca. 10l/min allowed its maintenance at a sufficiently low level (approx. 0.5%). The temperature in the chamber was between 23-25°C.

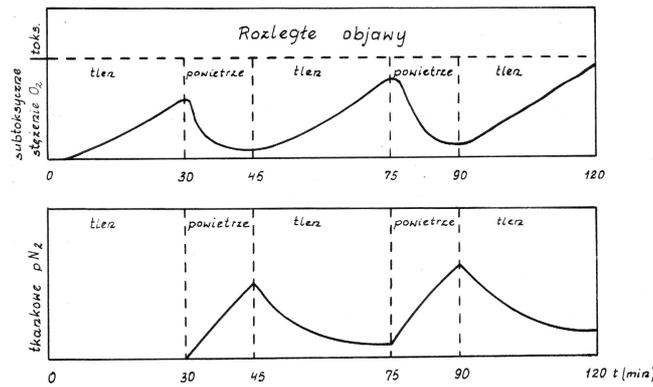


Fig.1. Experiment scheme (acc. to Lambertsen's modification).

The animals were observed during the entire exposure period. The time of occurrence of the first symptoms of oxygen toxicity was noted down along with their form and intensity. The development of toxicity syndrome in the tested animals is illustrated by fig. 2 and tab. I.

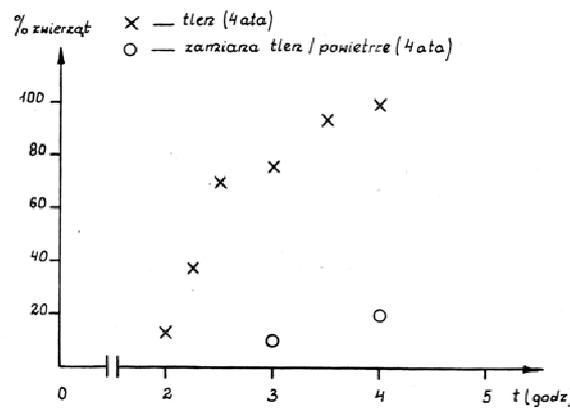


Fig.2. The relationship "concentration – time" in the development of oxygen toxicity symptoms in test animals.

Tab. 1

The development of symptoms in animals both breathing only with oxygen and those subjected to changes of the oxygen/air content.						
Time++/breathing gas	2h	2h15,	2h30,	3h	3h30	4h+ /
Oxygen	13%	38%	70%	76%	94%	100%
Oxygen/air	-	-	-	10%	10%	20%

+ / The experimented was not extended over 4 hours.

++ / For the group with interrupted exposures the "time" refers to the period on oxygen minus the intervals with the use of air.

Upon experiment completion the animals were removed from the chamber and terminated by breaking the continuity of the spinal cord at C<sub>2</sub> level, which was followed by dissection and collection of the lungs for the purpose of histopathological examination (performed by J. S. Meyer, M.D.).

## RESULTS

In animals subjected to uninterrupted hyperbaric oxygenation at 4 ata the first toxicity symptoms were observed after 2 hours, which was also noted in the experiments carried out by other authors [4,10], as well as in the previous proprietary tests [2]. The symptoms consisted of changes in the animals' behaviour; anxiety, and, finally, in gradually intensified convulsions starting with the hind legs and progressing to the torso and forelegs.

Though the convulsions weakened periodically over the time of the exposure, the general condition of the animals worsened, until eventually they lay on their side breathing heavily, showing no response to external stimuli. In the experiment involving continuous oxygen exposure this occurred in 100% of test animals.

The examination of the lungs of these animals revealed vast morphological lesions consisting in the occurrence of large atelectatic foci, plethora, foci of oedema in alveoli and alveolar septa and haemorrhages, mainly in the marginal areas of the lung lobes.



In the interrupted experiment scheme: oxygen – air, the first spasmodic changes were observed as late as after 3 hours and affected only one animal. After the lapse of 4 hours similar convulsions were noted in a second rat and the condition of both animals at this time of the experiment was similar to the condition characterising all animals in the control group.

The other animals after this period remained active, responded to external stimuli and revealed no symptoms comparable to those in the control group.

Morphological examination of the lungs of animals from this group indicated small extravasations into the lumen of the alveoli or alveolar septa and some oedemic fluid in the lumen of alveoli.

The lungs of the remaining animals of this group revealed good lung aeration, narrow interalveolar septa and average blood-flow in the parenchyma. Detailed morphological analysis of animals from this group became a separate subject of reference [8].

## DISCUSSION

An increase in the animals' tolerance to hyperbaric oxygenation, by use of interrupted exposure, depends on the proper selection of both the duration of oxygen exposures and interruptions at a given oxygen pressure. The experimental scheme applied in the research facilitated a delay in the occurrence of oxygen toxicity symptoms, resulting in their significant weakening and, most of all, it reduced oxygen toxicity to 20% of test animals as opposed to 100% of the animals from the control group with the same oxygen pressure (see tab.I.).

The adoption of any given scheme of exposures and interruptions is to a great extent dependent on the conditions of the hyperbaric oxygenation. It is known that in laboratory animals oxygen-induced lung injury (the

Lorrain Smith effect) is dominant in the clinical picture at lower oxygen pressures (usually below 3 ata), whereas central oxygen toxicity (the Paul Bert effect) is more common during short-term exposures above 4 ata pO<sub>2</sub> [4,5,6,9,10,11].

However, the above division is not rigid and allows concurrence of different forms of central oxygen toxicity for limiting oxygen pressures, with the dominance of one of them being dependant on co-existing factors (physical effort, temperature). This refers both to humans and animals, as according to Morgan [9], despite the lack of sufficient comparisons, it seems that the variations in the species sensitivity are not greater than individual variations.

We adopted the value of oxygen pressure of 4 ata due to the fact that it is close to the separation line of the two different effects of oxygen toxicity counting for the harmful effect of so high pO<sub>2</sub> values in all respects. Similar pressure values also constitute the upper limit adopted by hyperbaric medicine, and only slightly lower pressures are adopted in diving practices during decompression.

It is believed that morphological lesions in the lungs of rats appear as early as functional changes in people, at least within the lower oxygen partial pressures, and subjective changes were observed in people already after the lapse of 3 hours with a pO<sub>2</sub> of 2 ata.

All this encourages us to seek further methods of prolongation of safe exposures to increased oxygen pressures, although we are still far from fully understanding the observed phenomena.

The adopted experiment scheme: 30 minutes of oxygen – 15 minutes of air resembles the Lambertsen's scheme [6]: 30 minutes of oxygen – 10 minutes of 7% O<sub>2</sub> + nitrogen at 3 ata, and the Penrod's scheme [10]: 30 minutes of oxygen – 10 (or 5) minutes of air at 3 ata.

According to Lambertsen, improved results may be expected already with a 10-minute interruption following a 30-minute exposure. The 15-minutes adopted by us was expected to provide even better results, which was more or less confirmed with the consideration of differences in evaluation criteria.

It seems that after taking into account the different applied schemes [4, 6, 10, 13] it is possible to adopt a generalised principle that the introduction of interruptions lasting at least 30% of former oxygen exposure results in a significant increase in system tolerance to hyperbaric oxygenation. Reductions in oxygen concentration (e.g. to 7%) during such interruptions appears to further enhance this effect [6].

What requires separate revision is the presumable protective mechanism of the applied intervals. A relatively long latency period of oxygen toxicity with moderate hyperbaric oxygenation, as well as a quick return to the norm by lowering pO<sub>2</sub> has been proven on numerous occasions, both in diving and clinical practice.

The application of interruptions in hyperbaric oxygen exposures is expected to prevent the development of clinical toxicity symptoms as the toxicity barrier determined by the relationship "concentration-time" would not be exceeded, and general exposure time would be significantly extended (fig.1).

The fact of such a quick remission even after a symptomatic oxygen toxicity event is proven with the results of tests performed on animals quoted by Morgan [9].

According to Penrod [11] nitrogen plays an important role in the protective effect of air interruptions, as it fills the collapsed pulmonary alveoli and for a certain time brings back their ventilating function. This opinion was challenged by Dickerson [13] who believed that the prevailing lesion in pulmonary oxygen toxicity is not obstructive but rather compressive atelectasis due to pleural effusion, which would reduce the protective role of nitrogen.

The significance of nitrogen during the interruptions in hyperbaric oxygen exposures was also doubted by Wright [13], who during the intervals subjected animals to pure oxygen at the pressure of 200 mm Hg of the air and did not note a difference in the animals' survival rate.

Based on this, he drew a conclusion that the fact of implementation of a period with low oxygen partial pressure may per se have a favourable effect, irrespective of the presence or absence of nitrogen. However, it appears that the results of those tests performed in the conditions of atmospheric pressure should not be transposed onto the conditions of hyperbaric oxygenation.

The Lorrain Smith effect may also be a result of the unfavourable effect of hyperbaric oxygen on the factor maintaining proper surface tension of pulmonary alveoli (surfactant). This factor, a fat-protein compound, forms the lining of

pulmonary alveoli in normal conditions and reduces their surface tension thus preventing their collapse in the situation of reduced volume.

Thus, the presence of nitrogen, periodically reducing the concentration of oxygen contained in the alveoli, may express a favourable anti-atelectatic effect. This role may also be emphasised by the fact that in the observed clinical picture of oxygen toxicity the prevailing symptoms were those of ventilatory failure, which was also observed by Penrod [11]. Therefore, periodic air exposures could have prevented progressing lung injury, not only due to the decrease of pO<sub>2</sub> but also the presence of nitrogen.

## CONCLUSIONS

- The application of a 15-minute air interruption following a 30-minute oxygen exposure at 4 ata facilitates a delay in the occurrence of oxygen toxicity symptoms and significantly reduce incidence among animals.
- Morphological lesions in the lungs of animals subjected to interruptions in hyperbaric oxygen exposures tend to be much less susceptible to oxygen toxicity than animals subject to continuous exposures.

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