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ANALYSIS OF SPECIES CHARACTERISTICS OF LABORATORY ANIMALS IN REACTION TO HYPERBARIC ENVIRONMENTAL CONDITIONS

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ABSTRACT

The aim of this work is to determine the dynamics of nitrogen saturation in small laboratory animals. Nitrogen was chosen as a model gas in this study because of its availability and characteristics, as it is not metabolised and is subject to passive diffusion. By subjecting different species of animals to hyperbaric exposures of increasing time and pressure, the study aimed to identify how rapid a decompression was possible to achieve an outcome that saw 50% of the animals surviving the ensuing acute decompression sickness.
The basic parameters of hyperbaric exposure - pressure and time - made it possible to describe the saturation phenomena on the basis of partial saturation periods and to show whether a small animal organism can be considered as a single compartment model.
Keywords: decompression sickness, small laboratory animals, survival rate of 50%, species specificity.

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INTRODUCTION

Previous reports on nitrogen release from the human body and large experimental animals were based on the determination of the rate of excretion following administration of oxygen or a gas mixture containing no nitrogen [1,5,7,10,14,17-19,22,24,26,29,30,31,40]. Due to the inert gas distribution process in the system, this method cannot be used for small animals.

As early as the 19th century, various animal species were used to study the influence of the hyperbaric environment and the impact that exposure had on their organism once the pressure was reduced. The studies were carried out using different pressures, exposure times, decompression methods and breathing mixes. Nevertheless, it proved difficult to interpret the results obtained even on a single animal species, not to mention the comparison and evaluation of the degree of risk of decompression sickness of different species.

In most cases, researchers used one, very rarely several species of animals and did not aim at analysing species properties in the reaction of the system to conditions of overpressure. One of the most frequently used species in hyperbaric studies is the rabbit [6,18,24, 25,27,28,37,39], or guinea pig [3,6,7,35], whereas rats [1,2, 4-13,19,29,30,33,34,35,38,39,41,42] were the most frequently studied species [1,2,4-13,19,29,30, 33,34,35,38, 39,41,42]. Hamsters and mice were also used in research [2,3,5,6,21,25,29,31,32].

On the basis of the review of the studies, it can be concluded that the sensitivity of the animal species concerned was similar as regards toxic effects of oxygen [5,7,8,10,14,18,19,19,21,36), narcotic effects of inert gases [2-4,23,24,31,40], sensitivity to density of the breathing mixture and CO2 concentration [28], while there were significant differences in the susceptibility of each animal species to decompression sickness owing to variations in their body's ability to remove gas.

In the description of the symptoms of pressure disease in animals, the nomenclature adopted in the evaluation of decompression cases in humans [16-20] was used. The most frequently induced type of acute form of decompression sickness was characterised by neurological symptoms and pulmonary form with acute circulatory and respiratory insufficiency. However, symptoms of the first ChC type (joint and muscle pain – "bends") were observed only in large animals.

In the conclusions, the authors stressed that the obtained results cannot be considered as an attempt to explain the onset of decompression sickness in humans, nor can they be used to interpret the results of experiments with humans, or seek analogies with other animal species.

It would appear that despite an extensive bibliography on hyperbaric exposures on small experimental animals, no research had been carried out to determine the saturation dynamics expressed in periods of half saturation and to analyse the results differentiating these values for particular species.

OBJECTIVE

The aim of the study was to determine the survival rate of animals close to 50% at the lowest pressure level for each species of tested animals.

MATERIAL AND METHODS

The research was conducted on 5 animal species:

- rabbits – 24
- guinea pigs – 37
- rats – 37
- hamsters – 41
- mice – 47

These were mature animals of both sexes. The animals were treated with air hyperbaria in a pressure chamber with a capacity of 200 dm^3 and in a chamber with a capacity of 8 dm^3. The pressure was increased at a rate of 1-3 atm/minute to the assumed values. Decompression was carried out continuously for one minute on all the examined animals. During the experiments the temperature in the chamber was maintained within the range from 18 to 22^{o}C. Ventilation of the chamber was carried out periodically, in order not to exceed 1% of CO_2 concentration. During exposure, the number of the smallest animals in the chamber did not exceed 4 specimens. The determined parameters:

- survival threshold (pressure – exposure time),
- survival threshold for p=9 atm (exposure time).

Tab. 1.

The values of pressure and saturation time of a mouse's organism with nitrogen determining the survival threshold at one minute decompression.

Exposure	Weight	Average weight	Number	Fallen stock	Product f1x' 1	Deviation from mean value x' 1 - M	Squares of deviations x' 1 - M2	The product of the squares of deviations and numbers f1x' 1 - M2	Saturation pressure atm	Saturation time min	Survival rate
1	2	3	4	5	6	7	8	9	10	11	12
1	22,30 25,40 23,80	23,83	3	0	71,50	-1,27	1,61	4,84	13,8	20	
2	26,20 27,00 22,00	25,07	3	0	75,20	-0,03	0,001	0,003	13,6	25	
3	26,30 25,00 24,60	25,30	3	0	75,90	+0,2	0,04	0,12	13,4	30	
4	27,10 25,00 23,20	25,10	3	0	75,30	0,0	0,0	0,0	13,2	25	
5	24,20 23,60 27,20	25,0	3	0	75,0	-0,1	0,01	0,03	13,0	25	
6	22,80 25,60 24,20	24,20	3	0	72,60	-0,9	0,81	2,43	12,8	25	-
7	26,50 25,00 24,50	25,33	3	0	76,0	+0,23	0,053	0,159	12,6	20	-
8	24,40 26,20 26,50	25,70	3	0	77,10	+0,6	0,36	1,08	12,2	30	-

Tab. 1. cont.

The values of pressure and saturation time of a mouse's organism with nitrogen determining the survival threshold at one minute decompression.

9	27,00 22,50 26,30	25,27	3	2	75,80	+0,17	0,029	0,087	12,1	35	+-
10	25,60 24,80 26,60	25,66	3	2	77,0	+0,56	0,314	0,941	12,0	35	+-
11	24,60 24,80 25,40	24,93	3	2	74,80	-0,17	0,029	0,087	12,0	30	+-
12	23,80 23,20 26,40	24,46	3	2	73,40	-0,64	0,410	1,229	12,0	25	+-
13	23,40 22,70 27,00	24,37	3	1	73,10	-0,73	0,533	1,599	12,0	40	+-
14	25,60 25,40 25,80 24,40 26,70 26,50 26,20 26,60	25,9	8	5	207,20	+0,80	0,64	1,92	12,4	30	+-

The survival threshold for mice is characterised by pressure and time values of p=12.4 atm and t=30 minutes.

Tab. 2

The values of pressure and saturation time of a hamster organism with nitrogen determining the survival threshold for one minute decompression (columns as in the previous table).

1	2	3	4	5	6	7	8	9	10	11	12
1	65,25 70,00 68,50 65,00	67,19	4	0	268,75	-1,47	2,16	8,64	7,6	60	+
2	70,20 64,00 65,00 65,20	66,10	4	0	264,40	-2,56	6,55	26,21	7,6	70	+
3	65,30 70,40 68,00 58,80	65,63	4	0	262,50	-3,03	9,18	36,72	7,6	80	+
4	65,50 63,60 65,00 70,20	66,07	4	0	264,30	-2,59	6,71	26,83	7,6	90	+
5	65,70 62,00 68,40 67,20	65,83	4	1	263,30	-2,83	8,01	32,03	7,8	90	+-
6	65,00 68,20 58,20 61,25	63,16	4	1	252,65	-5,50	30,25	121,00	8,0	90	+-
7	68,50 80,00 71,25 68,40	72,04	4	1	288,15	+3,38	11,42	45,70	8,1	80	+-
8	65,20 80,40 81,25 64,50 68,70 80,20 70,00 68,20 70,00 68,80 72,40 80,30 81,20	73,16	13	7	951,15	+4,50	20,25	263,25	8,1	90	+-

The survival threshold for hamsters is characterised by pressure and time values of p=9.1 atm and t = 90 minutes.

Tab. 3

The values of pressure and saturation time with nitrogen for rats determining the survival threshold in relation to one minute decompression (columns as in the previous table).

1	2	3	4	5	6	7	8	9	10	11	12
1	243 230	236,5	2	0	473,0	10,39	107,95	215,90	6,4	90	+
2	235 246 260	247,0	3	0	741,0	+0,11	0,01	0,03	6,4	120	+
3	265 233	249,0	2	0	498,0	+2,11	4,45	8,90	6,6	90	+
4	250 268 220	246,0	3	0	738,0	-0,89	0,79	2,38	6,6	120	+
5	264 260 258	260,67	3	0	782,0	+13,11	171,87	515,61	6,6	130	+
6	246 254 250	250,0	3	0	750,0	+3,11	9,67	29,01	6,8	90	+
7	220 270 248	246,0	3	0	738,0	-0,89	0,79	2,38	6,4	130	+
8	240 260 238	246,0	3	0	738,0	-0,89	0,79	2,38	6,8	130	+
9	220 225 246	230,33	3	0	691,0	-16,56	274,23	822,70	7,0	130	+
10	220 270	245,0	2	0	490,0	-1,89	3,57	7,14	6,7	120	+
11	238 256	247,0	2	2	494,0	+0,11	0,01	0,02	7,6	130	-
12	220 267 240 250 260 245 248 272	250,25	8	5	2002,0	+3,36	11,29	90,32	7,2	130	+-

The survival threshold for rats is characterised by pressure and time values of p=7,2 atm and t = 130 minutes.

Tab. 4

The values of pressure and saturation time with nitrogen for guinea pigs determining the survival threshold for one minute decompression (columns as in the previous table).

1	2	3	4	5	6	7	8	9	10	11	12
1	372 378 285 292 383	342,0	5	0	1710	-11,37	129,28	646,38	6,2	160	+
2	290 282 374 367 378	338,2	5	0	1691	-15,17	230,13	1150,64	6,3	130	+
3	286 287 358 378	325,0	4	4	1300	-28,37	804,86	3219,43	7,0	180	-
4	380 386 375 350	372,75	4	3	1491	+19,38	375,58	1502,34	7,0	135	+-
5	365 350 358 388	365,25	4	4	1461	+11,88	141,13	564,54	7,0	120	-
6	367 384 366 372	372,25	4	4	1489	+18,88	356,45	1425,82	7,2	140	-
7	373 364 368 372 386 365 280 386 283 375 370 364	357,15	12	7	4286	+3,79	14,36	172,37	6,4	160	+-

The survival threshold for guinea pigs is characterised by pressure and time values of p=6,4 atm and t = 160 minutes.

Tab. 5

The values of pressure and saturation time of a rabbit system with nitrogen determining the survival threshold at one minute decompression (columns as in the previous table).

1	2	3	4	5	6	7	8	9	10	11	12
1	3400 3450	3425	2	0	6850	+45,56	2075,71	4151,43	3,2	180	+
2	3200 3500	3350	2	0	6700	-29,44	866,71	1733,42	3,4	180	+
3	3360 3310	3335	2	0	6670	-44,44	1974,91	3949,82	3,4	240	+
4	3280 3510	3395	2	0	6790	+15,56	242,11	484,23	3,6	240	+
5	3420 3460	3440	2	0	6880	+60,56	3667,51	7335,02	3,6	270	+
6	3400 3260	3330	2	0	6660	-49,44	2444,31	4888,63	3,7	270	+
7	3370 3290	3330	2	0	6660	-49,44	2444,31	4888,63	3,8	240	+
8	3400 3380 3420 3280	3405	8	4	13620	+25,56	653,31	2613,24	4,0	270	+-
9	3380 3360	3370	2	2	6740	+14,44	891,13	1782,26	4,2	270	-

The survival threshold for rabbits is characterised by pressure and time values of p=4,0 atm and t = 270 minutes.

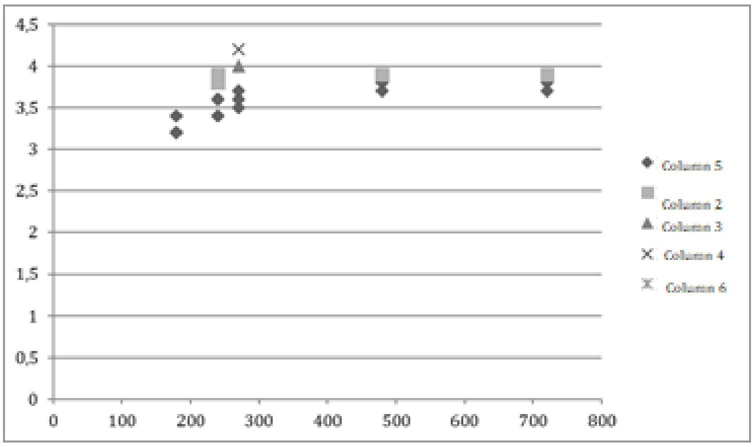

Fig. 1 Principle for determining a survival rate of approximately 50% for an animal at the longest exposure time using the example of a rabbit.

Figure 1 shows in the example of a rabbit a method of experimental determination of "critical" parameters of air hyperbaric exposure, i.e. values that would correspond to a 50% survival rate of the animals. "Critical" parameters were used to determine the longest required exposure time at the lowest pressure, because only in this case is it possible to determine and compare T 0.5 max for particular animal species. The determination of this value was one of the basic objectives of the study. The exponential character of the saturation phenomenon led to a considerable differentiation of time intervals. The correctness of choice for the survival of animals was confirmed by an increase in mortality at a higher pressure than at the survival threshold (50%) with the same exposure time and survival at a lower pressure but extended saturation time.

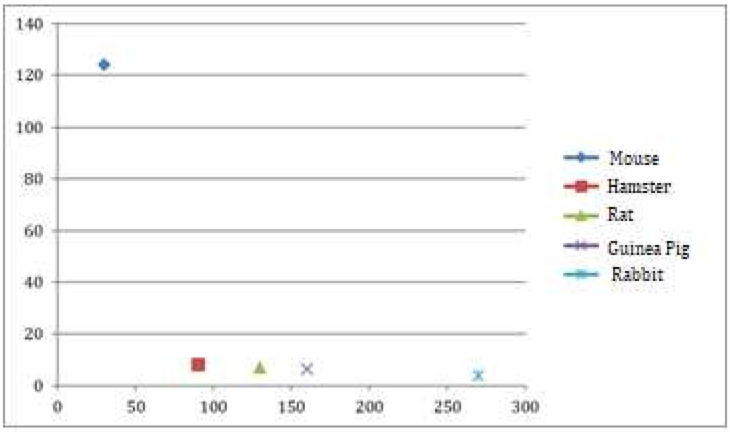

Fig. 2 Distribution of critical pressure and time values for particular animal species.

Figure 2 shows, on an arithmetic scale, the critical pressure and time values determining survival rates of approximately 50%. It indicates that the lowest exposure pressure ending in one minute decompression is tolerated by rabbits (4.0 atm), followed by guinea pigs, rats, hamsters and mice - up to 12.4 atm. The required exposure time, at which approximately 50% of the animals die, is the longest for rabbits and amounts to 270 minutes. For the other species it is reduced in the following order: guinea pig, rat, hamster, mouse.

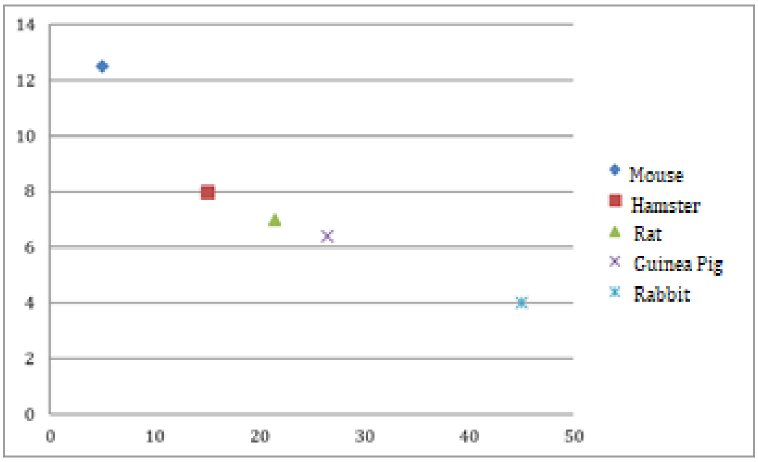

Fig. 3 Distribution of critical values of pressure and the longest saturation half-life.

Figure 3 shows in an arithmetic scale the distribution of survival values close to 50% as a function of pressure and the longest saturation half-life of 5 animal species. The saturation half-life values for individual species range from 5 minutes for mice to 45 minutes for rabbits.

The effect of high-pressure during breathing with air results in nitrogen saturation of the animal's body. The saturation in passive transport conditions takes place on an exponential basis and after six half-lives it reaches 98.5%. This allows to calculate T0.5 max for each animal species on the basis of the exposure times obtained. These values are shown in Figure 3, and the distribution determined by the T0.5 max values is analogous to Figure 2, discussed previously. The classification of the individual animal species in Figures 2 and 3 indicates the dependence of the values determining survival rates close to 50% on body weight.

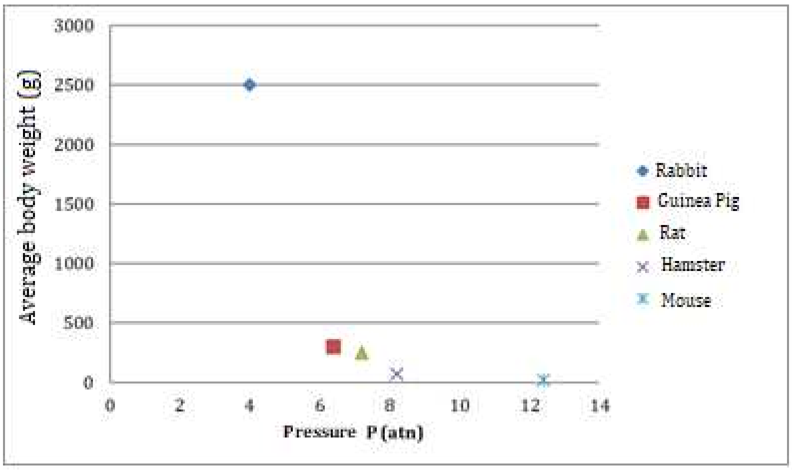

Fig. 4 Distribution of average body weight and critical saturation pressure values.

Figure 4 shows the distribution of tolerable pressures as a function of average body weight of individual animal species on a semi-logarithmic scale. It shows that the amount of the tolerated pressure remains dependent on the body weight. The rabbit with the highest mean body weight /3,450 g/ tolerates the lowest blood pressure /4.0 atm/, the mouse with the lowest mean body weight /24.5 g/ - the highest /12.4 atm/. The representatives of other animal species are included in the intermediate values of "linear" distribution.

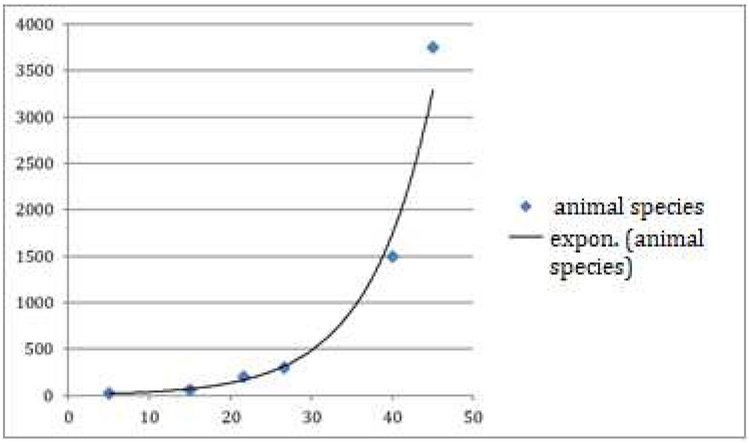

Fig. 5 Distribution of average body weight and longest saturation half-life.

Figure 5 shows the distribution of the longest empirically determined half-life of saturation as a function of the body weight of individual animal species on a semi-logarithmic scale.

The graph shows that the value of the longest saturation half-life is directly proportional to the body weight. The highest value of saturation half-life /45 minutes / was determined for rabbits /3,450 g / and the lowest value / 5 minutes / for mice /24.5 g/. The other animal species were found to be within the "linear" distribution values.

Based on the above, the relationships between the average body weight of the animals and the "critical" exposure pressure are presented.

Figures 4 and 5 show a clear correlation between body weight and the pressure and time values determining survival rates close to 50%.

Tab. 6

Critical values of the saturation time of a rabbit's system with nitrogen determining the survival threshold at a constant saturation pressure = 9 atm.

Exposure	Body weight of researched animals	Average body weight	Experimentally established critical values of exposure time	Survival rate	Observed symptoms	Comments
1	3,370		10 minutes	-	Fallen within 5 minutes from exposure	
2	3,460	3,388.33	9 minutes	-	as above	
3	3,490		8 minutes	-	as above	
4	3,420		7 minutes	-	as above	
5	3,330		6 minutes	+-	as above	Time zone indicating animal survival rate close to 50%
6	3,260		5 minutes	+	Survives with „bends" symptoms	

Tab. 7

Critical values of the saturation time of a guinea pig system with nitrogen determining the survival threshold at a constant saturation pressure = 9 atm.

Exposure	Body weight of researched animals	Average body weight	Experimentally established critical values of exposure time	Survival rate	Observed symptoms	Comments
1	340,0	341,87	40 minutes	-	Fallen within 5 minutes from exposure	
2	335,0		30 minutes	-	as above	
3	345,0		25 minutes	-	as above	
4	350,0		20 minutes	-	as above	
5	370,0		20 minutes	-	as above	
6	310,0		16 minutes	+-	Animal survives with severe symptoms of decompression sickness	Time zone indicating animal survival rate close to 50%
7	325,0		14 minutes	+-	Animals present „choke" symptoms	
8	360,0		10 minutes	++	Animals survive with decompressions sickness symptoms	

Tab. 8

Critical values of the saturation time of rats with nitrogen determining the survival threshold at a constant saturation pressure = 9 atm.

Exposure	Body weight of researched animals	Average body weight	Experimentally established critical values of exposure time	Survival rate	Observed symptoms	Comments
1	245 260	244,06	40 minutes	-	Fallen within 5 minutes from exposure	
2	255 220		35 minutes	-	as above	
3	267 225		30 minutes	-	as above	
4	240 238		28 minutes	-	as above	
5	248 254		27 minutes	-	as above	
6	232 246		26 minutes	+-	Animal survives presenting „choke" symptoms"	Time zone indicating animal survival rate close to 50%
7	235 243		25 minutes	+	„Choke" symptoms subside ca. 1 hour from exposure completion	
8	246 250		20 minutes	+	„Bend" symptoms lasting ca. 45 minutes after exposure completion	

Tab. 9

Critical values of the saturation time of three small laboratory animal species with nitrogen determining the survival threshold at a constant saturation pressure = 9 atm.

No.	Species	Number of animals used in experiment	Average body weight	Saturation time t_1^x	Saturation time t_2^x
1	Rabbit	6	3,388.33	38	6
2	Guinea pig	21	344,09	45	14
3	Rat	16	244,06	53,2	20

t_1^x - Critical saturation time values resulting in 50% survival of animals obtained using single-exposure procedures;

t_2^x - value of saturation time obtained experimentally in part two of the experiment.

Tables 6 - 9 show the distribution of body weight and critical saturation time values, which were empirically determined at a constant saturation pressure = 9 atm for three species of laboratory animals (rat, guinea pig, rabbit). The graph shows that the saturation time is inversely proportional to the body weight of the examined animals and is t=20 min for a rat with a body weight of 250 g, and t=6 min for a rabbit with a body weight of 3,450 g. The value of the saturation time for guinea pigs is within the intermediate values of the "linear" distribution.

On the basis of the discussed results it can be concluded that the saturation of small experimental animals with nitrogen is determined by the following longest half-lives of T0.5 max: mice- 21, 6 minutes, guinea-pigs- 26, 6 minutes, rabbits- 45 minutes, which is illustrated by the following equations of nitrogen distribution dynamics.

- ✓ rabbit $AtN_2 = A/1 - e - 0{,}0154\ t/$
- ✓ guinea pig $AtN_2 = A/1 - e - 0{,}0260\ t/$
- ✓ rat $AtN_2 = A/1 - e - 0{,}0340\ t/$
- ✓ hamster $AtN_2 = A/1 - e - 0{,}462\ t/$
- ✓ mouse $AtN_2 = A/1 - e - 0{,}1386\ t/$

The value of half-lives presented in the above equations is closely related to the average body weight. Therefore, exposure was carried out at a constant pressure of 9 atm. The results of the study indicate that for short periods of saturation, the use of only one exponent, even for small laboratory animals, is erroneous because the saturation process does not include periods shorter than T0.5 max (not determinable with a single exposure type). It follows from the above that the saturation process of four species of tested animals is described by the following equations:

- ✓ rabbit AtN_2 = A/1 - e -knt/+...A/1 - e -0,0154 t/ where kn 0,0154
- ✓ guinea pig AtN_2 = A/1 - e -knt/+...A/1 - e -0,260 t/ where kn 0,0260
- ✓ rat AtN_2 = A/1 - e -knt/+...A/1 - e -0,320 t/ where kn 0,0320

DISCUSSION OF RESULTS

It was assumed that the developed model of the course of nitrogen saturation of small laboratory animals should take into account the saturation (degree of saturation) of the longest absorption area in various species of laboratory animals [6,11-13,20,31]. According to the terminology used in the literature, the areas are referred to as tissues or compartments.

The volume of dissolved gas is a function of pressure and time. On the basis of this phenomenon, it can be shown that the lower the saturation pressure, without exceeding the critical threshold for a given species, the longer should be the required saturation time to reach the saturation level.

Considering the organism of the animals tested as a unit in which the gas is uniformly distributed (theory of homogeneous saturation) in the total volume, we are able to predict how long it will take for the animals concerned to show signs of decompression sickness at a fixed pressure for all tested species [1,5,9,15,21,25-27,32-37,41,42].

Symptoms of the course and severity of the pressure disease would therefore be a criterion for evaluation. The basic assumptions of the experimental model had to be based on the lowest possible exposure pressure and the longest time after which the tested animals develop symptoms of decompression sickness.

In order to achieve reproducibility of results with the lowest possible error for particular animal species, a single decompression time was established regardless of the threshold values of pressure and time. It was assumed that at one minute decompression it would be possible to induce symptoms of decompression sickness in the smallest examined animal species representatives, i.e. hamster and mouse.

Using the assessment criterion based on the symptoms of decompression sickness, a choice was made between inducing the first or second type of the disease in animals. The first type of decompression sickness was easy to induce in larger animals, inter alia, during the so-called "decompression titrations". [6,17,18,20]. However, it is very difficult to induce this type of disease in small animals. Therefore, in the assessment of the degree of saturation of animals with nitrogen, acute form of decompression sickness disease with mortality of observed animals close to 50% was assumed as an indicator of comparable full system saturation.

Theoretically, there were two possibilities of proceeding. The first was to start an experiment with high pressure values, causing 100% mortality, and a gradual transition to lower values in search of pressure and time values determined by a survival rate close to 50%.

The second is to start experimenting with low pressures, with gradual increase of pressure and prolongation of exposure time. The second method was chosen on account of lower losses of experimental animals.

The obtained results showed a correspondence of survival values close to 50% within individual species and a significant inter-species variation. Critical parameters (saturation pressure and time) indicate linear distribution. The reliability of the obtained results was confirmed by statistical analysis carried out with the use of the least squares method. Critical saturation times obtained by this method for particular species of animals could determine saturation in a single compartment model, assuming that this model has high probability properties or the longest saturation area in a non-single compartment system. Mathematical description of the above phenomenon depending on the function of the cardiovascular system can be presented in the original Haldane's formula [14,17].

The formula shows that the system will reach balance in the range of 98.5 per cent after six saturation half-lives. The logic is presented using the example of a rabbit. At the same time, this example is an explanation of the mathematical proof of the value of the saturation half-life.

The first method:

$S = (1-0,5)^n$; 1-0,015 = 0,985; S_6 = 270min. n=6
$0,5^6$ = 0,015; S_6 = 0,0985; 270: 6 = 45 min

The second method:

$A_t = a/1 - e^{-kt}/$
$T_{0,5} = \frac{1n2}{k}$
$k = \frac{0,693}{T0,5}$
0,693: 45 = 0,0154; k = 0,0154
$A_{45} = A/1 - e^{-0,0154 \times 45}/ = A/1 - e^{-0,693} / = A/1 -0,5/ = 0,5$
$A_{270} = A/1 - e^{-1x270}/$; k=?
$A_{270} = A/1 - 0,985/$
A_{270} = 0,015; if the value of e^{-x} = 0,015 to x = 4,2
hence 4,2: 270 = 0,0154 = k
after conversion
$T_{0,5}$ = 45 min.

This allows to convert the experimentally established criteria to a comparable saturation time value for each animal species, i.e. saturation half-life.

The thus calculated values are shown in Fig. 3. The distribution of these values is linear and the saturation of the largest laboratory animal - rabbit is 45 minutes, and of the smallest animal - mouse - 5 minutes.

This kind of relationship induced the search for relationships between body weight and the length of saturation half-life and tolerated pressure. The data presented on a semi-logarithmic scale showed the existing correlation resulting from the linear distribution. This led to further research. 24-hour exposure of rabbits at p=3.8 atm and 8-hour and 12-hour exposure of a cat at p=4.6 atm confirmed the correctness of the determined maximum saturation time - despite repeated prolongation of the exposure time, the animals survived. Results obtained on rabbits after 12-hour exposure at 3.8 atm showed that after 270 minutes, i.e. the maximum time used to determine the survival threshold, the animal body remained saturated to no additional extent. This test result verified the correctness of the previously determined maximum half-life, which in a rabbit amounted to 45 minutes.

How in a given model can this dependence between the semi-saturation intervals and tissue perfusion rates be demonstrated? The low solubility of nitrogen in the aqueous phase (water; solution - 0.0145 at 37°C, blood - 0.0141 at 37°C) allows to assume a constant relationship between the concentration of the gas in the respiratory mixture and "alveolar air" regardless of the breathing phase.

$V_I=V_E=V_{tid}$

This suggests that cyclic alveolar ventilation should be presented as

$\tilde{V}_A=N/V_{tid} - V_D/$

of a continuous flow in which the gas is balanced between the parallel streams of the breathing mixture and the pulmonary circulation blood. The diffusion balance and the percentage of clearance (% C1) are the result of the following relationships:

$$\%C1 = \frac{\dot{V}out}{\dot{V}in} = \frac{\dot{V}A * Fa}{\dot{V}A*FA*\dot{Q}*FA} = \frac{1}{1+\dot{Q}/\dot{V}A*R}$$

$$\frac{\dot{Q}}{VA} - \frac{5\ 1/min}{4\ 1/min}\ R= 0{,}0145$$

$$\%C1 = \frac{1}{1+5/4R}$$

This allows (for N2 - 0.98) to assume that the nitrogen pressure in the alveoli and the arterial blood of the pulmonary circulation can be considered equal.

Based on the above, in the mathematical description of the 1st order kinetic processes expressed in the formula:

$$T_{0,5} = \frac{1n2}{k}$$

The constant of k loss should be dependent on the size of tissue perfusion [14,17].

$$k = \frac{\dot{Q}bit\ Sb}{Vti\ Sti}$$

where:
$\dot{Q}_{bti}$ – blood flow through the tissue (cm^3/min/cm^3)
S_b – gas solubility in blood (cm^3/cm^3)
Sti – gas solubility in tissue (cm^3/cm^3)
V_{ti} – tissue volume (cm^3)
By substitution to formula 2.1 we receive:

$$T_{0,5} = \frac{0{,}693\ Vti\ Sti}{\dot{Q}bti\ Sb}$$

$$\dot{Q}bti = \frac{0{,}693\ Vti\ Sti}{T0{,}5\ Sb}$$

Value $\frac{Sti}{Sb}$ is the tissue/blood gas partition coefficient.

The determination of the value of tissue perfusion as burdened with too much error was abandoned because in the single-compartment range the mean value for the partition coefficient should be used, while in the case of a two-compartment model, the "longer" tissue would be characterised by the partition coefficient with a higher value.

In order to determine the saturation model presented by the examined animals, hyperbaric exposures of rabbits, guinea pigs and rats at a constant pressure of 9 atm, i.e. the size exceeding the critical pressure value of the mentioned animal species, were carried out. However, it was not possible to perform such tests on hamsters and mice.

For these animals, it was impossible to significantly exceed the critical pressure value due to the non-negative effects of high partial pressure of oxygen, nitrogen and high density of the breathing mixture. The animals would be exposed to central oxygen poisoning, nitrogen anaesthesia and hypercapnia, which would not be without impact on the results of the studies [2-4,7,8,10,18,19,21,22-24,27,30,35,38,39,41].

The critical values obtained for the saturation times of rabbits, guinea pigs and rats were several times shorter than those calculated assuming that the small animal body corresponds to the one-compartment model.

Due to simple pressure-time relationships, the one-compartment model omitted the justification for the discrepancy between the calculated and the actual time of exposure [15].

The exposure at 9 atm pressure clearly indicates that faster saturation of the system was conditioned by the participation of at least one other absorption area (compartment) reaching the equilibrium state much earlier than it would be indicated by the values of half-lives determined and discussed in the first part of the experiment.

Based on the results obtained, the saturation of small experimental animals with nitrogen, using the method of elaborated hyperbaric exposures, is presented in table 10 below.

Tab. 10

The saturation of small experimental animals

Rabbit	$AtN_2 = A/1 - e^{-knt}$ / +... $A/1-e^{-0{,}0154}$/, where kn >0,0154
Guinea pig	$AtN_2 = A/1 - e^{-knt}$ / +... $A/1-e^{-0{,}0260t}$/, where kn >0,0260
Rat	$AtN_2 = A/1 - e^{-knt}$ / +... $A/1-e^{-0{,}0320t}$/, where kn >0,0320
Hamster	$AtN_2 = A/1 - e^{-knt}$ / +... $A/1-e^{-0{,}0462t}$/, where kn >0,0462
Mouse	$AtN_2 = A/1 - e^{-knt}$ / +... $A/1-e^{-0{,}1386t}$/, where kn >0,1386

Using the method of air hyperbaric exposure to determine the dynamics of the process of saturation with nitrogen, it was found that during decompression the survival of individual species of animals used in the study, depended on the determined saturation half-lives. It results from the following conditions: formation of gas bubbles / separation of gas phase / and further growth of gas bubbles takes place in a defined period of time. At the same time, excess gas in tissues caused by the difference in pressure is carried away by blood and removed from the system.

Thus, the shorter the half-life of saturation, the greater the tissue perfusion and the more effective the equalisation of the concentration of gas dissolved in the tissue. Such a state makes it possible for the animal to tolerate higher pressures while decompression time is the same (1 min.).

CONCLUSIONS

- The interpreted results indicate that the nitrogen saturation of rabbits, rats and guinea pigs does not follow the single-compartment model.
- The relationship between the saturation half-life (T0.5) of a rabbit, guinea pig, rat, hamster and mouse system and body weight on a half-logarithmic scale is of linear character.
- During rapid decompression (within one minute) following air hyperbaric exposure, the pressure drop tolerated by the animal is greater the lower the species-specific body weight.
- The determined critical parameters deviate from the values determined for humans to such an extent that they are not useful when determining decompression patterns for divers.

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