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THE EFFECTS OF SOME HYDROPHOBIC GASES ON THE PULMONARY SURFACTANT SYSTEM

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1 Decompression from exposures to raised ambient pressure of sulphur hexafluoride, carbon tetrafluoride, hexafluoro-ethane and nitrous oxide results in the formation of dense foam and pulmonary oedema.

2 The degree of pulmonary oedema produced is dependent on the exposure pressure, although the exposure time required is short in comparison to tissue saturation times.

3 The effect is not prevented by atropine, ephedrine or hydrocortisone.

4 The effect is also produced *in vitro* by saturated solutions of halothane, chloroform and ether.

5 It is suggested that the mechanism of action is physical with the physico-chemical factor involved being a differential partition of these gases within the surfactant: membrane complex.

Introduction

This study has been primarily concerned with the effects of decompression from raised ambient pressures of sulphur hexafluoride (SF_6), although comparative experiments have been done with carbon tetrafluoride (CF_4), nitrous oxide (N_2O), hexafluoroethane (C_2F_6) and nitrogen (N_2). The study of SF_6 was initiated because of two unusual observations made some years ago (Lever, 1969). Firstly, on decompression from an exposure to SF_6 either no, or only scanty, bubble formation could be detected *post-mortem*, even when death of the animal had occurred. Secondly a white or pink foam was observed in the mouths of some animals after, but not before, decompression. It appeared that this foam could have been the cause of death in some instances, by causing the animals to become anoxic. Death after decompression without apparent bubble formation was contrary to most accepted theories of the role of a separated gas phase in the aetiology of decompression sickness (Elliott & Hallenbeck, 1975). Therefore it seemed that SF_6 must possess another property, linked to the production of foam, which caused death probably due to some action in the lungs. The experiments in this work have been concerned with measurements of dry/wet lung weight ratios, which are used as an index of pulmonary oedema.

As SF_6 has been used for respiratory studies and carbon tetrafluoride has been suggested for use in submarine escape procedures, an examination of any possible pulmonary effect of these gases was considered desirable. In addition the anomalous behaviour of SF_6 in decompression studies affords the possibility of investigating the mechanism of decompression sickness, in particular the role of gas bubbles.

Methods

Charles River, male CD-1 mice, in the weight range 23 to 27 g were used for all *in vivo* experiments. The mice were allowed free access to food and water before their use in an experiment and were never used until at least one day after their arrival at the laboratory.

The mice were exposed to pressure in a 2.9 litre decompression chamber, fitted with two viewing ports in the side wall. Before compression the chamber was flushed with O_2 and after termination of the decompression the chamber was, normally, again flushed with O_2. The level of CO_2 was kept constant throughout the exposure by means of a fan stirring the chamber gases and soda lime scrubbers placed inside the chamber at the start of each experiment. After decompression the mice, unless otherwise stated, were kept under observation for 30 min before being removed from the chamber. The number of mice from each group which had died was recorded and the lungs removed from all the mice. A total of 4 groups of 5 mice were exposed at each pressure studied. The lungs were weighed wet, freeze dried and then weighed dry. The control for all of the experiments consisted of a group of 21 mice which had not been

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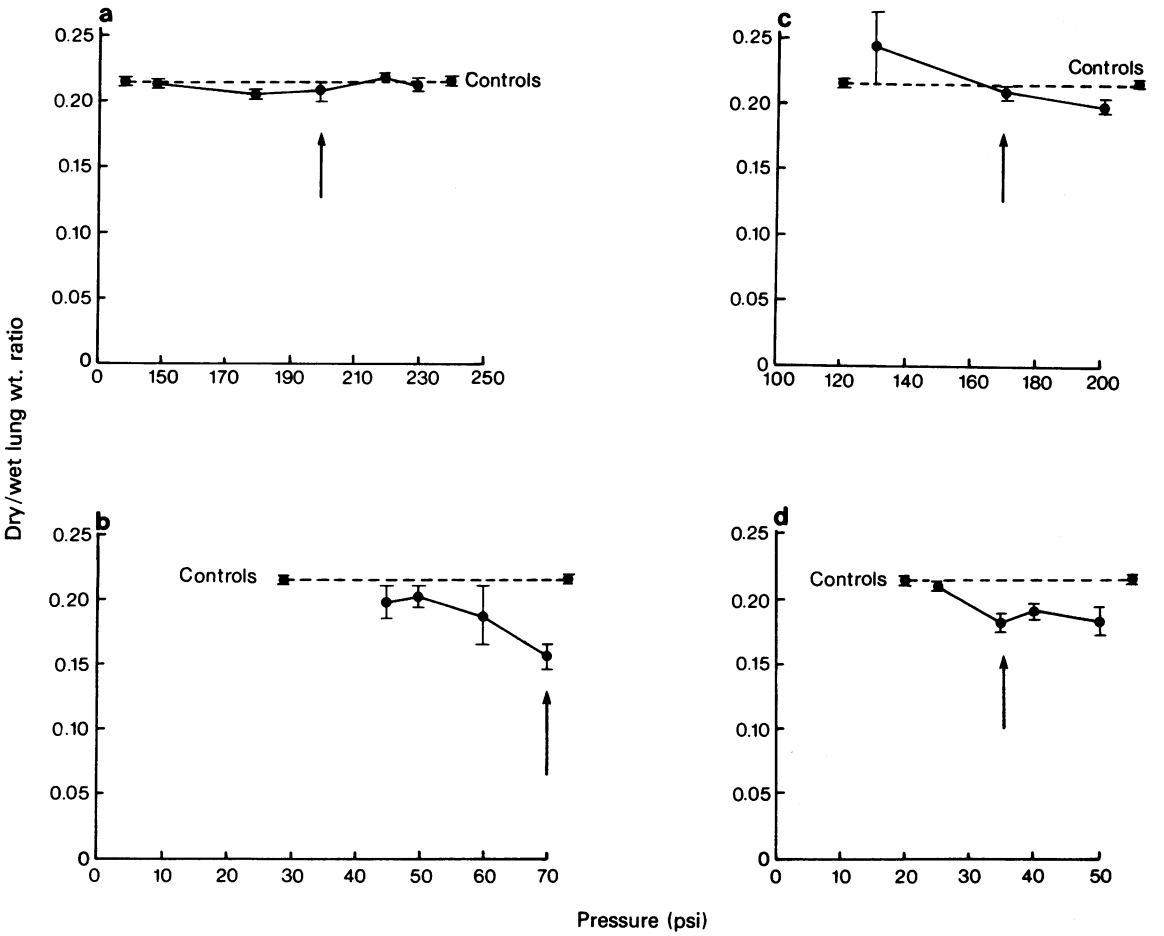

Figure 1 Dry/wet lung weight ratios following saturation exposures and rapid decompression with (a) nitrogen, (b) sulphur hexafluoride, (c) carbon tetrafluoride and (d) nitrous oxide. Arrow indicates LD_{50} exposure pressure. Vertical bars represent s.e. mean.

exposed to pressure and whose dry/wet lung weight ratios were determined in the same way.

For *in vitro* experiments with guinea-pig lung, guinea-pigs weighing 400 to 750 g were stunned and exsanguinated. The excised lungs were suspended inside a glass vessel by means of a plastic tube tied into the trachea, with the other end of the tube open to the atmosphere. Gas could be introduced into the glass vessel to raise the ambient pressure and hence produce a pressure difference across the lung. Depending on the pressure range, the pressure was measured with either a water or a mercury manometer. The anaesthetic solutions were injected into the lungs with a syringe, via the supporting tube. The volume of fluid returned was in turn measured by directing this tube into a 5 cm^3 burette.

Results

Experiments involving saturation exposures to various pressures of N_2, SF_6, CF_4 and N_2O

The groups of 5 mice were exposed for 90 min to various pressures of N_2, SF_6, CF_4 and N_2O, this time of exposure having been shown by Lever (1969) to be sufficient for saturation. These exposures were followed by a rapid (15 s) decompression and a 30 min interval in O_2 at atmospheric pressure. The LD_{50}s i.e. the pressure of each gas which on decompression after a 90 min saturation exposure led to 50% mortality, were calculated by the technique of probit analysis according to the method of Finney (1952). These, together with the alterations in dry/wet

lung weight ratio are shown in Figure 1 (a, b, c and d). Statistical analysis, by Student's t test, showed that the dry/wet weight ratios after N_2 exposure did not differ significantly from the controls. However, the dry/wet ratios after SF_6 exposures were significantly different from both the controls ($P < 0.001$) and from the N_2 values ($P < 0.001$). The dry/wet ratios after the higher dose of CF_4 differed statistically from the control level ($P = 0.025$), whilst the dry/wet ratios after all but the lowest dose of N_2O differed significantly from the control value ($P < 0.001$). It can be seen from Figure 1 that the level of oedema depends on the exposure pressure.

The incidence of visible foam was 80% after exposure to 70 psig SF_6 and 20% after exposure to 200 psig CF_4; after N_2O exposures foam was only observed in 3 cases, once at each of the three higher exposure pressures. This lack of visible foam after N_2O exposure is probably due to the fact that with the three higher exposure pressures the mice all died before decompression, together with the fact that the magnitude of the pressure change is small compared to the SF_6 and CF_4 exposures. Foam was never observed after the N_2 exposures. With all these exposures, lungs in which foam was observed were always oedematous, except that after some N_2O exposures, foam was not visible although the lungs were oedematous as measured by the dry/wet lung weight ratio. In appearance, the lungs of the mice in which foam was apparent were distended and resistant to manual collapse, indicating that the compliance of the lung had been destroyed.

The mean weight of fluid appearing in lungs with a significantly low dry/wet ratio (calculated from the change in lung dry/wet ratio and weight) was 0.28 ± 0.02 g. This is approximately 1% of body weight, and in a 75 kg man would correspond to about 0.75 litre of fluid.

Exploratory experiments at a single pressure of C_2F_6 showed that after a 90 min exposure to 70 psig the dry/wet lung weight ratio was significantly lower than the control value, indicating a high degree of pulmonary oedema. Furthermore foam was observed in the lungs and trachea of all the group of mice used in this experiment.

Although no experiments using helium (He) were done specifically to test for a pulmonary effect, foam has never been seen after any of the experiments with helium carried out over a number of years. Furthermore there are no reports in the literature of any such effect.

Experiments to investigate the effect of time on the appearance of foam and oedema

Two separate factors have been investigated; (a) the time of exposure to 70 psig SF_6 before decompression and (b) the time between decompression and killing the animal, with oxygen being replaced by air in the post-decompression period. The dry/wet lung weight ratios for these two series of experiments are shown in Figure 2.

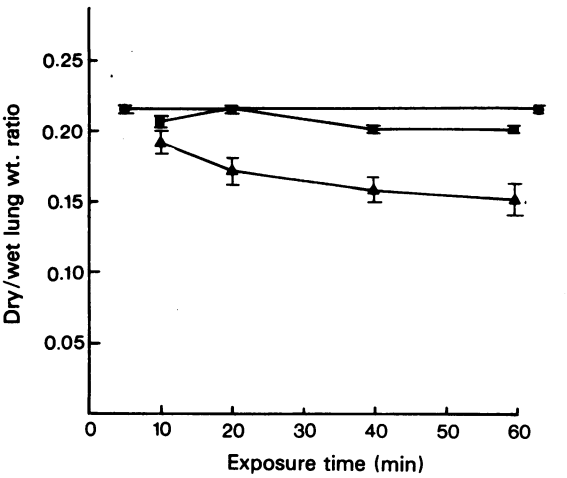

Figure 2 The effect on dry/wet lung weight ratio of varying the time of exposure to 70 psig sulphur hexafluoride, with (▲) and without (■) a 30 min post decompression period in 1 ATA pure O_2. (●) Control values. Vertical bars represent s.e. mean.

The experiments first showed (Figure 2, lowest curve, with a 30 min post-decompression period in pure O_2) that the intensity of the oedema increased with duration of exposure but also that a significant effect was already present after only 10 min exposure to SF_6. Although the levels of oedema after 20, 40 or 60 min exposure do not differ significantly there is a general trend of increasing oedema with increasing time, with the maximally effective exposure time about 60 min.

If the animals were killed immediately after decompression (Figure 2, upper curve) much smaller degrees of oedema were found, although those with 40 and 60 min exposure times were still statistically significant ($0.001 < P < 0.01$). Despite the reduction in oedema, the incidence of visible foam was the same as in the previous experiments. This indicates that very little fluid is needed for foam to be produced.

Since it was possible that the O_2 to which the mice were exposed after decompression could facilitate the production of oedema, two further experiments were carried out. First, mice were exposed to oxygen at atmospheric pressure for up to 5 h; no difference in dry/wet lung weight ratios from the controls was detected. Secondly, the O_2 in the post-decompression interval was replaced by air for 30 min. This increased the incidence of death during the 30 min interval

(from 14% to 60%); the incidence of foam was unchanged (80%); the degree of oedema was less. The reduction in oedema was attributed to the early death of the mice; 60% died rapidly after decompression showing all the signs of asphyxia. This early death would reduce the time during which the circulation was intact and oedema fluid able to accumulate. For this reason, and since the incidence of foam was unchanged, it was concluded that the oxygen exposure did not itself have any damaging effect on the lung, but served primarily to counter the effect of the foam in the airways and protect the animals from an early anoxic death.

Effect of atropine, ephedrine and hydrocortisone on the production of foam and oedema

In the light of other known causes of pulmonary oedema, experiments were performed to test the possibilities that the appearance of the foam (a) was due to a neurogenic (vagal) action (Cameron & De, 1949), or (b) involved some vascular change leading to grossly increased filtration pressure in pulmonary capillaries, or (c) was in the nature of an acute inflammatory response, by testing the effect of pretreatment with atropine (5 mg/kg and 1 mg/kg), ephedrine (5 mg/kg) or hydrocortisone (10 mg/kg). The hydrocortisone was given as hydrocortisone sodium succinate and the 10 mg/kg corresponds to 7.48 mg/kg of the free alcohol. These drugs were all given intraperitoneally 15 min before compression. An exposure to 70 psig SF_6 for 40 min followed by rapid decompression was given to each of the 4 groups of 5 mice used for each dose of drug. In no case was any reduction in the fall of the dry/wet lung weight ratio observed. The only difference noted was that after pretreatment with atropine or with ephedrine the incidence of death was increased from the usual value of 14% to 100%.

The insensitivity of the pulmonary oedema to any of these drugs suggested, therefore, that the action of SF_6 is due to some direct physical interaction.

In vitro *experiments to study the action of solutions of some volatile anaesthetics on excised guinea-pig lungs*

The volatile anaesthetics were chosen for this study because a physical property they have in common with SF_6, unlike N_2, is a high fat:water partition coefficient. In these experiments known volumes of saturated solution of the anaesthetics halothane, chloroform and ether in saline were injected into an excised guinea-pig lung; a small external pressure was applied to the lung and the curves of pressure against volume of solution ejected were plotted. These experiments, in addition, tested the need for an intact circulation, with the possibility of the involvement of a specific cardio-vascular mechanism in the production of the foam and pulmonary oedema.

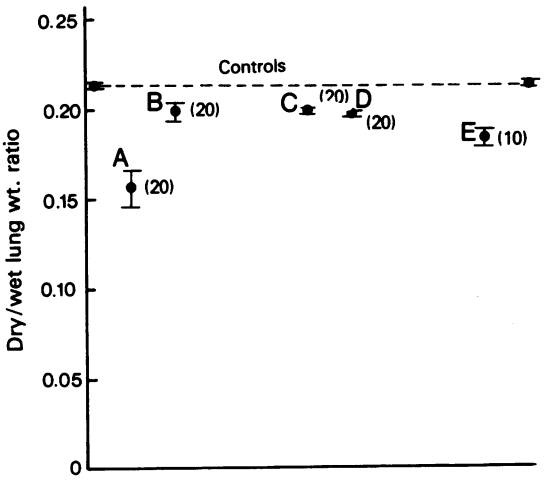

Figure 3 Importance of decompression in producing pulmonary oedema, as measured by a decrease in dry/wet lung weight ratio. The experimental conditions were as follows: (A) 40 min at 70 psig sulphur hexafluoride (SF_6) → rapid decompression → 30 min 1 ATA O_2; (B) 40 min at 70 psig SF_6 → rapid decompression; (C) 40 min at 70 psig SF_6 → 60 min at 70 psig air → rapid decompression; (D) 40 min at 70 psig SF_6 → 60 min at 70 psig air → rapid decompression → 30 min 1 ATA O_2; (E) 40 min at 70 psig SF_6 → 230 psig He for 10 min → decompression to 70 psig for 30 min → rapid decompression. Vertical bars represent s.e. mean.

It was found that saturated solutions of halothane, chloroform and ether all produced both the gross loss of compliance of the lungs and the production of foam that characterized decompression with SF_6. These results support the hypothesis that the mechanism of action is a direct physical interaction and make less likely any mechanism requiring an intact circulation. Further experiments with non-saturated solutions of the anaesthetics will be described separately.

Experiments to examine the role of decompression in the production of foam and oedema

In these experiments an attempt was made to distinguish between the effects of exposure to SF_6 and of decompression. The first series of experiments were designed so that the exposure to SF_6 and the decompression were separated in time. For this purpose a 40 min exposure to SF_6 was followed by replacement of the SF_6 with air; this was done by flushing the chamber with air at a rate of 67 l/min for 10 min (23 chamber volumes per min) keeping the pressure

constant at 70 psig. The mice were maintained in air at 70 psig for 60 min and were then rapidly decompressed, after which they were immediately removed from the chamber and killed (procedure C) or they were left for 30 min in O_2 at atmospheric pressure before they were killed (procedure D). The dry/wet lung weight ratios for both these experiments are shown in Figure 3, compared to the value for the control group (dotted line), and to values obtained in mice given either a 40 min exposure to 70 psig SF_6 followed by rapid decompression and a 30 min period in O_2 (A) or a 40 min exposure to 70 psig SF_6 followed by rapid decompression and immediate killing of the mice for examination (B). It is clear that there is a great reduction in the degree of oedema when the exposure and decompression are separated in time, although a small residual effect remained ($0.001 < P < 0.01$). This residual effect is perhaps a reflection of the long half time for elimination of SF_6, 25 min for whole body elimination from mice (Lever, 1969). The incidence of visible foam following these experiments was 35% when a period for accumulation of fluid was allowed before examination and 25% when no such period was allowed. These values compare to an incidence of visible foam of 80% for the exposure to SF_6 followed immediately by decompression. This low incidence of visible foam is consistent with the observation that little oedema occurs when the exposure and decompression are separated in time.

The second series of experiments were designed to separate the exposure and the decompression by superimposing a compression: decompression profile, using helium (He) for which there is no evidence of any pulmonary effect, onto an exposure to SF_6 which, if followed directly by decompression and immediate killing of the animal, would not lead to any significant oedema (B in Figure 3). This was achieved by exposure of the mice to 70 psig SF_6 for 40 min; increasing the pressure to 230 psig with He; holding this pressure for 10 min and then rapidly reducing the pressure to 70 psig (end partial pressures approximately P_{O_2} 0.4 atmospheres (ATA), P_{SF_6} 1.6 ATA, P_{He} 3.7 ATA); this pressure was then maintained for 30 min before rapid decompression after which the mice were killed immediately for examination. The value of the dry/wet lung weight ratio is shown in Figure 3 (E), and indicates that, in this case, a significant degree of oedema resulted ($P < 0.001$). This oedema must have been due to the helium decompression: since the mice were killed immediately after the final decompression, the possibility of the final decompression resulting in significant oedema can be excluded. In these animals the incidence of visible foam was 30%, suprisingly low when the measured degree of oedema is considered.

Finally, further evidence for the role of decompression was provided by a series of experiments consisting of a 40 min exposure to 70 psig SF_6 followed by a slow decompression, at a rate of 10 psi/min. In this case the dry/wet lung weight ratios did not differ significantly from the control value and the incidence of visible foam was reduced from 80% to 10%.

It is apparent from these experiments that in order to produce the full final effects of foam and oedema both exposure to SF_6 and a rapid drop in the pressure of SF_6 are necessary.

Discussion

The visible foam and oedema shown in these experiments, while related, are in practice distinct; the foam appears immediately after decompression but time is required for the oedema fluid to accumulate. The experiment with a superimposed helium decompression even suggested that the foam could be disposed of in some way while oedema formation was progressing. The degree of pulmonary oedema was found to be dependent on the exposure pressure and time. However, the exposure time required is short compared to the whole body saturation time. The foam was stable and resistant to Silicon anti-foam A, unless a protease such as trypsin was also present. It was concluded, therefore, that the foam consisted, as expected, largely of di-palmitoyl lecithin, (Pattle, 1958; Pattle & Thomas, 1961; Klaus, Clements & Havel, 1961). This was further supported by the similarity between the infra-red spectrum of the foam and that of synthetic di-palmitoyl lecithin.

The experiments suggest, that SF_6, and to lesser degrees C_2F_6, CF_4 and N_2O, cause some dissociation of the surfactant from the alveolar surface; that this mechanism is physical; and that for these gases the decompression is essential in producing the full final effect. The fact that saturated solutions of halothane, chloroform and ether can reproduce the effect *in vitro* supports the view that the mechanism is physical. A summary of physical properties which may be relevant is given in Table 1.

A complicating feature for any attempted correlation is the effect of death on the observed effect. Death after decompression resulting from cardiac arrest due to bubble formation will act to reduce the level of oedema. But with the three higher N_2O exposures respiratory death occurred before decompression. In this case a continuing circulation (Bock, 1913) with increasingly hypoxic blood could act to increase any oedema-producing effect of exposure to N_2O.

An assessment of the relative importance of the physical factors is attempted below.

By analogy with theories of decompression sickness (Lever, Paton & Smith, 1971; Hennessy & Hempleman, 1977) a correlation with lipid solubility or with

Table 1 Correlation between pulmonary oedema and certain physical properties of gases

Gas	*LD_{50} after saturation exposure: 15s decompression* (psig) (ΔP)	*% reduction in dry/wet lung wt. ratio. Measured at LD_{50}* (5)	*Olive oil:gas partition coeff. at 37°C* (3) Sf	*Water:gas partition coeff. at 37°C* (3) Sw	*Critical volume* (4) Vc	$Sf \times \Delta P$	$Sw \times \Delta P$	*Estimate of membrane expansion* $Sf \times \Delta P \times Vc$ E	*Estimate of differential solubility* Sf/Sw	$\frac{Sf}{Sw} \times \Delta P$	$E \times \frac{Sf}{Sw}$
SF_6	70[1]	27	0.3	0.005	198	21	0.35	4158	60	4200	24.95×10^4
C_2F_6	70[5]	17	0.13	0.004	224	9.1	0.28	2038	32.5	2275	6.62×10^4
N_2O	36[2]	13	1.4	0.44	97.4	50.4	15.84	4914	3.2	115	1.57×10^4
CF_4	170[2]	8*	0.072	0.005	140	12.2	0.85	1714	14.4	2448	2.47×10^4
N_2	203[1]	3*	0.075	0.015	89.5	15.2	3.05	1362	5	1015	0.68×10^4
He	217[1]	0‡	0.018	0.0096	57.3	3.9	2.08	223	1.9	412	0.04×10^4
Halothane			224	0.9	300						
Chloroform			265	4	239						
Ether			65	4.8	280						

* Not significant; ‡ taken to be 0% (see text).

(1) Daniels, 1974; (2) Lever, 1969; (3) Miller & Smith, 1973; (4) Reid, Prausnitz & Sherwood, 1977; (5) Present work.

lipid solubility × exposure pressure might be expected. Inspection of Table 1 shows such correlation to be poor, especially for the lipid solubility × exposure pressure. Alternatively over-hydration of pulmonary cells by gas-induced osmosis (Hills, 1972) during decompression may be responsible; but the expected correlation with aqueous solubility is absent. Furthermore, Halsey & Eger (1973) showed that such an osmotic gradient is overcome by a water shift of about 0.16% plasma volume, a hundredth of the observed effect.

In the absence of any correlation with a single factor, a number of further possibilities can be examined. The effect may be due to an expansion of the alveolar membrane caused by solution of the gases. This expansion will depend on the lipid solubility, the exposure pressure and a suitable factor related to the size of the molecule, such as the critical volume. Excluding N_2O, Table 1 shows a reasonable correlation between estimated expansion (E) and the degree of oedema ($r = 0.952$). On this basis N_2O would be expected to have a much larger effect than is observed. An alternative possibility is that the level of oedema is related to a differential expansion of the lipid and hydrophilic (possibly protein) parts of the surfactant:membrane complex. An estimate of this differential effect is given by the oil:water partition coefficient. This yields an excellent correlation ($r = 0.994$), again excluding N_2O. On this basis, the level of oedema observed after N_2O is 6.5 times greater than expected. This relationship, however, does not include any factor which might account for the observed effect of exposure pressure on the level of oedema. A simple product of differential solubility and exposure pressure gives a poor correlation. However, a good correlation is found by combining the terms for membrane expansion and differential solubility ($r = 0.940$), which also reduces the N_2O discrepancy; in the case of N_2O the observed effect is 2.2 times greater than expected.

Therefore, while recognising both the difficulties of making general statements from a small sample and the anomalous behaviour of N_2O, we conclude that the physico-chemical factor involved, acting together with the decompression, is probably a differential partition of these anaesthetic gases within the surfactant:alveolar membrane complex, leading to a weakening of the surfactant:membrane binding. The capacity of anaesthetics to disrupt membranes has long been known, exemplified by their ability to lyse red blood cells and we suggest that because of the looser binding of the surfactant to the alveolar membrane the latter may be disrupted more easily.

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