ALVEOLAR STABILITY OF ALTITUDE RAISED RATS

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Summary: On the lining of alveolar membrane of the lung presence of surfactant, a surface tension lowering agent which is a phospholipid in nature, is well established. A good correlation exists between pulmonary lecithin, the principal constituent of surfactant system and alveolar stability. The production of surface active material in the type II cells is oxygen dependent and is affected by hypoxia.

The chemical and physical nature of the surfactant in the lungs of the rats raised at high altitude has been studied in comparison with that of sea level control. Eighteen male adult rats raised at high altitude (3520 m) were used. Phospholipids (Phosphatidyl Choline, Phosphatidyl ethanolamine, Lysophosphatidyl choline, Lysophosphatidyl ethanolamine and Sphingomyelin) were estimated by thin layer chromatography, stability index by Pattle's bubble technique and dynamic surface tension have also been studied to assess the surfactant activity of the lung.

The results indicate that there was a decrease in lung surfactant as measured by chemical analysis. However, stability ratio measurements showed that there was very little change in the stability ratio (Sr) as the value of both groups lay in the normal range namely 0.6 to 0.9 hence the lungs of high altitude raised rats had normal alveolar stability. Surface tension values of alveolar lavage in altitude raised animals were also similar to those of normal rats.

It is concluded that the rats raised at altitude show a lowering of surfactant as estimated chemically but the stability ratio is not significantly altered to indicate alveolar instability. In the altitude rats lesser quantum of surfactant is adequate to maintain alveolar stability.

Key words: stability ratio surface tension phospholipids surfactant hypoxia

INTRODUCTION

The presence of an alveolar lining layer as an interface between the air and the alveolar surface is well established (14). The alveolar lining material or surfactant lowers the surface tension adjusted to the alveolar radius and thereby reduces the lung recoil. The unique elastic properties of the lung which permit the stabilization of alveoli at varying lung volumes during respiratory cycle are largely determined by this surface active lining layer coating the alveoli. This surfactant lining may subserve another function. It together with tissue forces and plasma osmotic pressure resists the effusion of plasma into alveolar spaces (3,9). The surfactant system has a high lipid content and phospholipid comprises 74% of the lipid. Cholesterol, triglyceride and unesterified fatty acids are present in lesser amount (20). Phosphatidyl choline has the greatest surface activity of the phospholipids but others most notable sphingomyelin and phosphatidyl dimethylethanolamine are also surface active (15,34). Since phosphatidyl choline (lecithin) is the most abundant alveolar lipid (55%

of total lipid) and has the greatest surface activity, it is logical to assign it the primary role in lowering surface tension. A good correlation exists between the amount of pulmonary lecithin and the tendency to collapse and atelactasis (2,3,7).

The continuous replacement of the phospholipids in the surfactant material of the alveolar surface required a high rate of phospholipid synthesis in the lung tissue (32,36). Hypoxia has a well marked effect on the biosynthesis of phospholipids due to the inhibition of oxidative energy production and depression of esterification of palmitate (23). Changes in alveolar gaseous environment may, therefore affect lipid metabolism and surfactant synthesis which may be reflected in alveolar stability (25,26).

Rats raised at altitude are exposed to hypobaric hypoxia since birth. The aim of the present study, therefore, was to assess the effect of hypoxia on alveolar stability by measuring the surface tension and quantitative phospholipid estimation.

MATERIALS AND METHODS

Adult male albino rats were used in this study. Albino rats were raised at an altitude of 3520 m above sea level. Eighteen rats were brought from high altitude to the laboratory at Delhi by plane. On arrival they were further exposed to the same hypoxic environment of corresponding altitude in an altitude chamber for 3 days before sacrifice.

An equal number of albino rats of approximately the same age and breed from DIPAS colony were used as sea level controls. The animals were divided into three groups: for chemical study, stability ratio and surface tension measurements.

Chemical analysis:

The rats were sacrificed by exsanguination via abdominal aorta after intraperitoneal injection of sodium pentabarbitone (40 *mg/kg* body weight). Lungs were dissected out and lavaged with 25 *ml* isotonic saline. Fluid was withdrawn, reintroduced five times to ensure maximum extraction of surfactant. The lung lavage was centrifuged at 300 g for 10 minutes to remove tissue elements. The supernantant was separated out and recentrifuged at 4°C at 2000 g for 60 mins. The sediment fraction was separated out. The supernatant was treated with an equal volume of chilled 25% TCA. The precipitate was centrifuged out and supernatant was discarded. Thus lavage fluid was separated into two surfactant fractions (i) the white sediment fraction representing lipid rich component and (ii) TCA precipitate fraction containing lipoprotein following the method of Fujiwara *et al.* (13). Lipids were extracted from these two fractions according to the method of Folch (12).

The lung tissue was homogenised in chloroform : methanol mixture (2 : 1 V/V) so as to give 10% W/V homogenate, final extract was obtained by the method of Folch (12).

Total lipids were analysed gravimetrically. Thin layer chromatography was done to fractionate individual phospholipids on silica gel G using Chloroform : Methanol : 7 M Ammonia 115: 45: 7.5 (V/V/V), as solvent (1). The phospholipid spots were visualised by exposing the TLC plate to Iodine vapours, spots of individual phospholipids were marked and scrapped off from the plate for estimation of phosphorus by the method of Bartlett (4). The values of phosphorus were converted into phospholipid values by the multiplication factor 25.

Stability ratio:

Estimation of alveolar stability has been done by Pattle's bubble technique (28). The rats were sacrificed by exanguination via abdominal aorta after intraperitoneal injection of sodium pentabarbitone (40 *mg/kg* body weight). Lungs were carefully excised and weighed. A small portion of inflated lung tissue was squeezed into a drop of aerated isotonic saline on a glass coverslip. The coverslip was then inverted on a cavity glass slide. To prevent evaporation the two were sealed together with a drop of saline. Measurements were made on bubbles initially 35-60 μ in diameter with a calibrated occular disc. A rough sketch of the bubbles were drawn on paper. After 20 mins the diameter of the bubbles was again measured and recorded. The alveolar stability was expressed as $(D2/D1)^2$. Mean sr of 20 bubbles in 4 batches of 3 each from widely separate areas of lung has been used as a measure of the state of the alveolar lining film.

Measurement of surface tension:

Surface tension of the lung lavage has been measured by the bubble technique. The experimental set up is shown in Fig. 1. Approximately 0.2 *ml* of the lavage fluid in a petri dish is allowed to be sucked by the capillary. The bubble is formed at the end of - capillary C of radius 0.095 cm by pushing air into the system with syrings S. The bubble is viewed through a travelling microscope and is measured by coinciding with concentric circles O drawn on an occular disc. The pressure inside the bubble was measured by a manometer containing Broadies fluid (sp. gr. 1.083). The radius. of the biggest ring was 0.2 cms while for a minimum ring it was 0.1 cm. The corresponding pressures were read on the manometer. Before starting the experiment the bubble formed was made to expand and contract several times. When the bubble was stable the values of P for corresponding decreasing values of radius were noted. The min. and max. surface tension was calculated by the formula $T=rp/4$. The pressure P is given by hdg. Where h is

manometric height, d is density of the manometric fluid and g is the acceleration due to gravity.

RESULTS

The body weight, lung weight and lung weight body weight ratio for the sea level controls and high altitude raised rats are given in Table I. The altitutde raised rats had a significantly higher lung weight ($P<0.001$). The lung weight/body weight ratio is also significantly higher in the altitude raised rats ($P<0.001$).

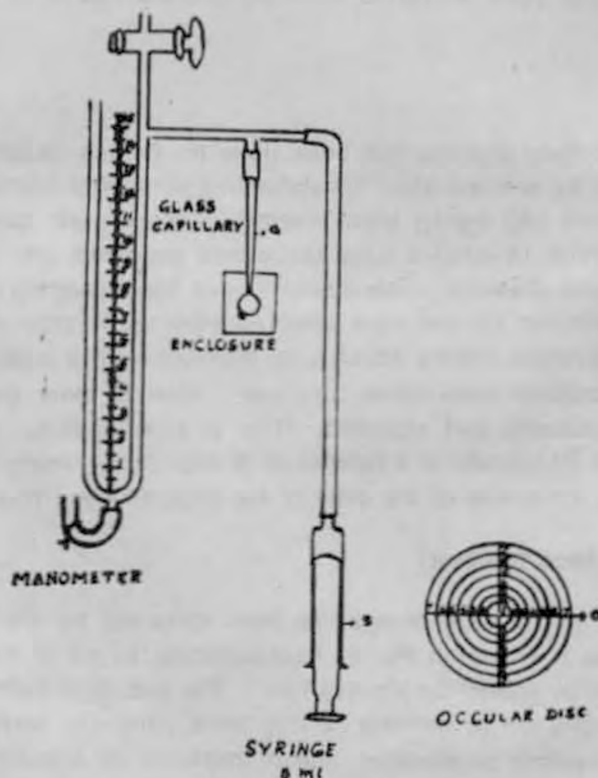


Fig 1: Appratus for measuring surface tension by bubble method.

Haematological picture;

The haematological parameters studied in the altitude raised rats and sea level control rats are given in Table II. The altitude raised rats had a significantly higher concentration of haemoglobin in their blood. The packed cell volume was significantly higher in altitude raised rats. An insignificant increase in RBC number was observed.

TABLE I: Body weight, lung weight and lung weight body weight ratios.

	Body weight (gms)		Lung weight(gms)		Lung weight/body weight Ratio x 10^{-3}	
	Control rats	*Altitude raised rats*	*Control rats*	*Altitude raised rats*	*Control rats*	*Altitude raised rats*
n	18	18	18	18	18	18
Mean	245.21±5.32	265.70±10.31	1.3286±0.0796	2.0011±0.1192	5.39±0.28	7.43±0.41
Significance	N.S.		P<0.001		P<0.001	

Values are mean ±S.E.M

TABLE II: Haematological picture.

	Control rats	*Altitude raised rats*	*Significance*
Haemoglobin gm/100 ml	13.88±0.30	16.20±0.84	P<0.05
P.C.V. %	47.67±0.76	53.60±0.68	P<0.001
RBC x 10^6 cu. mm	5.97±0.89	6.10±0.26	N.S
Platelets x 10^3	484 ±20.92	319 ±28.98	P<0.01

Values are mean ±S.E.M

The number of platelets in altitude raised rats was significantly low ($P < 0.01$).

Lung tissue lipids:

Total lipids, phospholipids, phosphatidylethenolamine, phosphatidyl choline, lysophosphatidylcholine and sphingomyelin fractions of lung tissue in sea level control and altitude raised rats are given in Table III.

TABLE III: Lung tissue lipids.

	Control rats	*Altitude raised rats*	*Significance*
mg/gm wet tissue			
TL	35.1450 ± 0.5330	30.4660 ± 0.4280	P < 0.001
TP	21.7341 ± 0.3334	19.6172 ± 0.4945	P < 0.01
PE	6.2119 ± 0.2209	6.2070 ± 0.3191	NS
PC	9.3494 ± 0.2137	7.2210 ± 0.1600	P < 0.001
Sph + LPE	0.7941 ± 0.0685	0.6781 ± 0.0488	NS
LPC	0.7649 ± 0.0433	0.5402 ± 0.0575	NS

Values are mean ± S.E.M

TL = Total lipids
TP = Total phospholipids
PE = Phosphatidyl ethanolamine
PC = Phosphatidyl choline
Sph = Sphingompelin
LPE = Lysophosphatidyl ethanolamine
LPC = Lysophosphatidyl choline

Total lipids and phospholipids in lung tissue of the altitude raised rats showed a considerable decrease ($P<0.001$) and ($P<0.01$) respectively. Phosphatidyl choline decreased by about 25% while the lysofraction though present in smaller quantity also showed a decrease. No change was observed in other fractions namely phosphatidyl ethanolamine and sphingomyelin.

Lung lavage lipids:

As mentioned earlier the alveolar surfactant system lavaged from the lungs was divided into two fractions (i) Sediment fraction and the (ii) TCA fraction. The total phospholipids in the sediment fraction were 50% of the TCA fraction. There was no change in the sediment fraction due to hypoxia in the altitude raised rats. In the TCA fraction the total lipids were significantly decreased ($P<0.05$). The decrease was mainly due to a decrease in the phosphatidyl choline ($P<0.001$) while an increase has been observed in phosphatidyl ethanolamine ($P<0.001$) and lysophosphatidyl choline ($P<0.05$). no change was observed in sphingomyelin. The results are given in Table IV.

TABLE IV: Phospholipids in lung lavage.

	TCA fraction			Sediment fraction		
	control rats	*Altitude raised rats*	*Significance*	*control rats*	*Altitude raised rats*	*Significance*
mg/gm wet tissue						
TP ..	1.0439±0.0626	0.8368±0.0339	P<0.05	0.4415±0.0360	0.4283±0.0218	N.S.
PE ..	0.1067±0.0097	0.1671±0.0085	P<0.001	0.0776±0.0087	0.0581±0.0033	N.S
PC ..	0.4905±0.0320	0.2070±0.0066	P<0.001	0.2711±0.0176	0.2996±0.0086	N.S
Sph .. + LPE	0.0779±0.0091	0.0820±0.0060	NS	—	—	—
LPC ..	0.0766±0.0108	0.1056±0.0072	P<0.05	—	—	—

Values are mean ±S.E.M

TP = Total phospholipids; PE = Phosphatidyl ethanolamine
PC = Phosphatidyl choline; Sph = Sphingomyelin
LPC = Lysophosphatidyl choline; LPE = Lysophosphatidyl ethanolamine

Alveolar stability ratio:

The stability ratio showed no significant decrease in altitude raised rats, 0.836 ± 0.0022 as compared to sea level control rats, 0.887 ± 0.0040. The normal range has been reported to be varying between 0.6 to 0.87 (17,28) and a value below 0.6 is considered to be associated with unstable alveoli (6,27).

Surface tension:

The surface tension (dynes/cm) values measured at different surface areas in sea level control and high altitude raised rats were, 55.27±0.435 and 54.76±0.869 (100% surface area), 45.64±0.468 and 45.00±0.727 (73.6% surface area), 39.26± 0.578 and 38.46±0.604 (51% surface area), 33.41±0.450 and 32.16±0.567 (32.6% surface area) and opening pressure (mb) were 2.439 ±0.042 and 2.3988±0.031. Thus there was no significant change in surface tension and opening pressure in sea level control and high altitude raised rats.

DISCUSSION

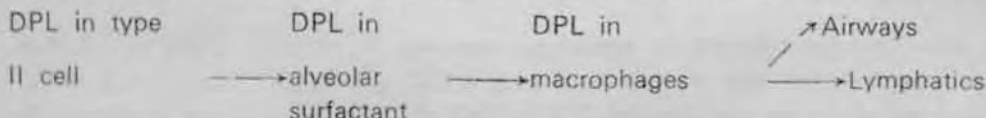
The altitude raised rats showed typical haematological picture of chronic altitude stress with increased haemoglobin, packed cell volume and red blood cells. The platelet

count in altitude raised rats was observed to be significantly less than in sea level controls. Altitude exposure in rabbits has been shown to decrease the platelet count due to increased sequestration in the lung suggesting increased trapping and destruction of platelets in the pulmonary vasculature (16, 18). Possibly the same mechanism is operative in altitude raised rats which affects platelet survival time.

The wet lung weight and lung weight body weight ratio was significantly higher in altitude raised rats. Bartlett and Remmers (5) demonstrated in young rats that exposure to hypoxia acted as a stimulus to alveolar proliferation and resulted in increased lung volume and alveolar surface areas in high altitude animals. Further, under low atmospheric pressure the expansion of the thoracic cage has to be more to create an inspiratory pressure gradient analogous to that at sea level. The increased expansion of thoracic cage in the young stimulates lung growth. Further Cunningham *et al.* (10) have also suggested possible limitation of lung growth by thoracic cage.

Chemical composition:

Naimark (24) postulated a simplified hypothetical scheme according to which there is normally a continuous production and removal of surfactant and its substituent dipalmitoyl lecithin (DPL). Lung activity incorporates precursors of DPL including palmitate, glycerol, glucose, choline and acetate in the type II alveolar cell (8,11,23,25). Intracellularly synthesized surfactant is then secreted on to the alveolar surface. The clearance of used surfactant is done by alveolar macrophages (29,30).



According to Naimark (24) the above diagram represents the process of a dynamic balance of the surfactant production. Our data showed a significant decrease in total lipids, total phospholipids, phosphatidyl and lysophosphatidyl choline fraction in the lung tissue of altitude raised rats as compared to sea level controls. Hypoxia has been shown to slow the rate of metabolism in several tissues due to decreased levels of ATP (21,22,31,33). Contrary to this basal and ADP mediated respiration remains unchanged. The observed decrease in phospholipids in lung tissue therefore, may be due to inhibition of lipid synthesis, which is oxygen dependent.

In the alveolar lavage in the TCA fraction a significant decrease was observed in phosphatidyl choline and phosphatidyl ethanolamine fraction of phospholipids. As mentioned above decreased synthesis of surfactant in type II cells was reflected in decreased surfactant in the lung lavage fluid of the altitude raised rats, the other reason for this decrease could be an alteration in the secretion rate. However, our data do not throw any light on this aspect.

The sediment fraction did not show any change in the total phospholipids and the constituent fractions.

Alveolar stability :

A decrease in the surfactant in the lung levage fluid would increase the surface forces in the alveoli. A poor stability of the alveoli would be expected in the altitude raised rats. But our data showed no change in the alveolar stability ratio measured by Pattle's bubble method. The value for sea level controls was 0.887 ± 0.044 as compared to 0.836 ± 0.002 for altitude raised rats. The normal range of stability ratio varies from 0.6 to 0.87 as shown by other workers (17,27,28). Only values of sr below 0.6 have been considered pathological (6). Our findings are in agreement with those of N wman and Naimark (26) who found no alteration of bubble stability ratio despite decreased synthesis and turnover of phospholipids in hypoxic situations.

Surface tension of lung lavage :

Studies on surface balance with alveolar surfactant spread over a large surface area show a decrease in surface tension with decrease in surface areas. In the first phase of compression to about 40% area the value of surface tension decrease to 33 dyne/cm. On further compression to 20% area the value of surface tension decreases to a value below 10 dynes (19). In the absence of a surface balance and an automatic recording device, we have measured surface tension by the bubble method where the compression could be done to 30% of the area. Hence our readings are confined to the first phase only. The maximum and minimum values of the surface tension did not differ in the altitude raised rats and sea level controls.

We have also measured the opening pressure required to blow a bubble at the tip of the capillary. The pressure is maximum when the bubble is hemispherical in shape and represents the pressure required to overcome surface force during inflation. Young *et al.* (35) have considered this pressure as a factor to explain 42% decrease in lung compliance at low transpulmonary pressure. This pressure expressed in mb also did not change in the altitude raised rats.

Hence we conclude that the rats raised at altitude show a lowering of surfactant as estimated chemically but the stability ratio is not significantly altered to indicate alveolar instability. In the altitude adapted rats lesser quantum of surfactant is adequate to maintain alveolar stability.

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