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13 ABSTRACT

To investigate basic mechanisms operative in high altitude pulmonary edema, pulmonary extravascular water volume (Q_{pevw}) was measured in 11 unanesthetized calves exposed to atmospheres equivalent to 12,000 and 16,000 feet. Measurements were made by a double indicator dilution technique at sea level and after continuous exposure of 24, 48, and 72 hours. Thirty-five duplicate measurements from 15 experiments yielded a test-retest reliability coefficient of 0.84. Data from 15 experiments were technically satisfactory after exposure of 24 hours; 13 experiments after 48 hours exposure; and 7 experiments after 72 hours exposure. After 24 hours exposure the mean increase in Q_{pevw} was 42.7 ml ($p < .025$); after 48 hours 79.3 ml ($p < .005$); and after 72 hours, 29.3 ml (N.S.). There was no significant difference in Q_{pevw} for the same duration of exposure at 12,000 feet and 16,000 feet. It is concluded that Q_{pevw} increases in the bovine lung after exposure to high altitude.

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SUMMARY

To investigate basic mechanisms operative in high altitude pulmonary edema, pulmonary extravascular water volume (Q_{pevw}) was measured in 11 unanesthetized calves exposed to atmospheres equivalent to 12,000 and 16,000 feet. Measurements were made by a double indicator dilution technique at sea level and after continuous exposure of 24, 48, and 72 hours. Thirty-five duplicate measurements from 15 experiments yielded a test-retest reliability coefficient of 0.84. Data from 15 experiments were technically satisfactory after exposure of 24 hours; 13 experiments after 48 hours exposure; and 7 experiments after 72 hours exposure. After 24 hours exposure the mean increase in Q_{pevw} was 42.7 ml ($p < .025$); after 48 hours 79.3 ml ($p < .005$); and after 72 hours, 29.3 ml (N.S.). There was no significant difference in Q_{pevw} for the same duration of exposure at 12,000 feet and 16,000 feet. It is concluded that Q_{pevw} increases in the bovine lung after exposure to high altitude.

INTRODUCTION

Pulmonary edema occurs sporadically in unacclimatized individuals exposed to altitudes greater than 10,000 feet and the impairment of lung function may cause severe incapacitation or death. Although patients have been studied by cardiac catheterization during high altitude pulmonary edema, the basic causitive mechanisms and the sequence of fluid accumulation in the lung have not been determined.

There is evidence that the pulmonary interstitial space serves as a reservoir for edema fluid in pulmonary edema caused by hemodynamic overload (1-3) and it has been noted that this fluid compartment is expanded before clinical manifestations of pulmonary edema appear (3). The development of an objective measurement that would indicate impending high altitude pulmonary edema would be of considerable value in elucidating the pathogenesis and in formulating a rational approach to the prevention of this condition. With this goal in mind we have measured pulmonary extravascular water volume (Q_{pevw}) by indicator dilution techniques in animals exposed to simulated high altitude in a hypobaric chamber. Our findings are presented in this report.

PROCEDURE

GENERAL

Eleven calves from local dairy stock were studied in 16 experiments. Their ages ranged from 7 days to 3 months and their weights ranged from 34 pounds to 170 pounds. The animals were anesthetized with halothane and radio-opaque catheters were inserted into the right jugular vein and right common carotid artery and advanced under fluoroscopic control to the right ventricle and aortic arch respectively. The animals were allowed to recover from the effects of surgery for 18 hours and then were transported to the low pressure chamber. In the chamber they were placed in a stanchion and duplicate indicator dilution curves were obtained at sea level. The animals were then placed in a small pen and the chamber evacuated to a simulated altitude of 12,000 feet or 16,000 feet. After 24 hours at altitude, the chamber was entered through an external lock and measurements were performed in duplicate. This procedure was repeated at 48 hours and 72 hours. After the 72 hour measurements were obtained, the animals were returned to sea level and, in most cases, were sacrificed. Figure 1 shows a calf in the stanchion. In the figure the anaerobic sampling device is connected to the indwelling carotid catheter and the mixture of radioactive indicators is being injected into the jugular catheter.

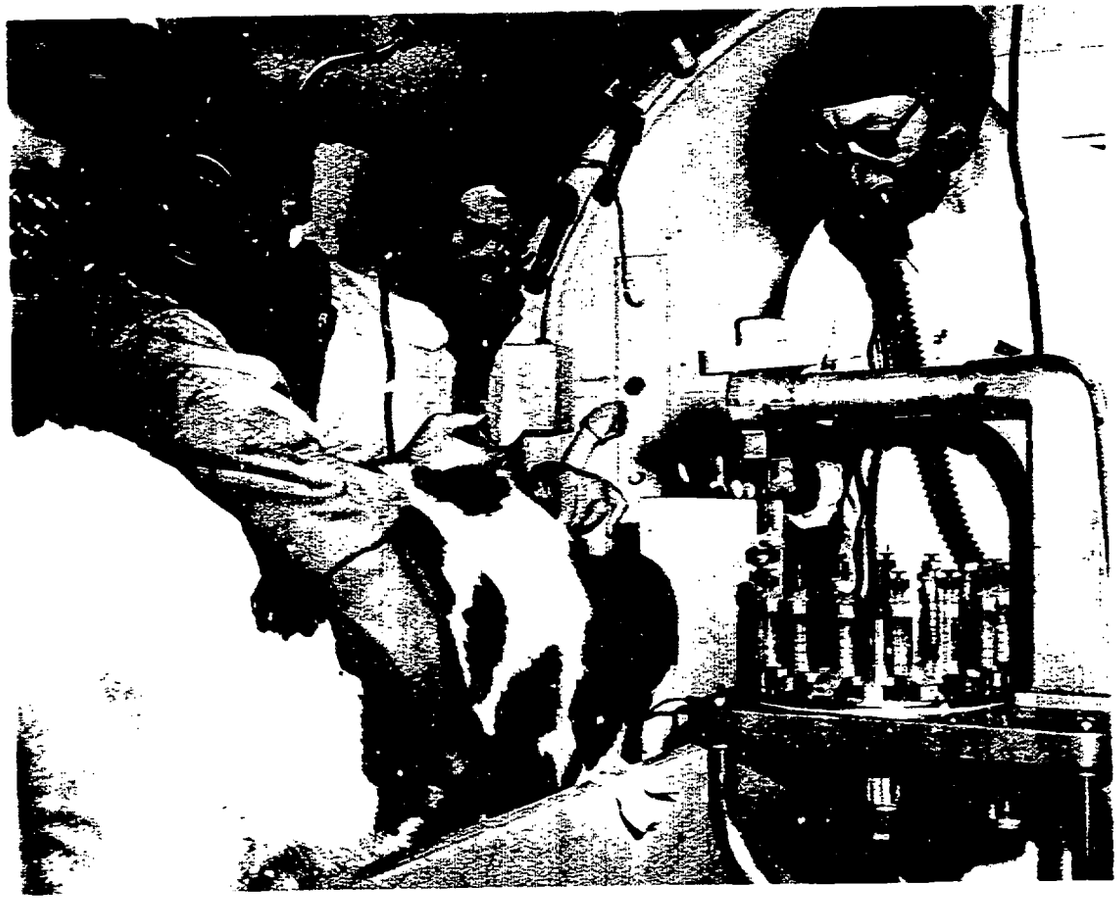

Figure 1

An experiment being conducted in the low pressure chamber. The calf's head and neck are supported by a stanchion. The carotid catheter is connected to the anaerobic sampling device and the solution containing the radioactive indicators is being injected into the venous catheter.

MEASUREMENT OF PULMONARY EXTRAVASCULAR WATER VOLUME

Albumin I^{131} and tritiated water in a solution of normal saline were prepared in a sterile syringe. This injectate contained approximately 7 μc of I^{131} and 300 μC of tritiated water. This solution was injected rapidly into the catheter that had been previously positioned in the right ventricle and at the time of injection a mark was made on a moving strip chart. After a delay of about two seconds the arterial sampling device (Fig. 1) was started and 19 fixed-volume samples were withdrawn from the indwelling aortic arch catheter. The dead space of the syringes was 0.3 ml; the sampling rate was 0.15 ml/sec; and the volume of the right ventricle catheter was approximately 0.7 ml. No attempt was made to control the animal's respiration during the sampling period.

PREPARATION OF SAMPLES AND MEASUREMENT OF RADIOACTIVITY

A 0.5 ml aliquot of each sample was placed in a 5 ml tube and the I^{131} activity determined in an Ames Gammacord scintillation detector. The counting times were chosen to give less than 5% standard error. The injectate was diluted with whole blood to give counts in the same range as the highest sample counts. After I^{131} activity was determined, the same aliquot was prepared for the tritiated water analysis by adding 3 ml absolute alcohol, mixing well, and centrifuging for 10 minutes at 3000 rev/min. One ml of alcohol-water supernatant was added to 10 ml of a scintillation mixture made of Naphthalene; 2,5-diphenyloxazole (PPO); 2, 2-P-phenyl enebis (5-phenyloxazole) (POPOP); methanol; ethylene glycol; and dioxane. The preparation was then counted in a Packard Tri-carb model 3310 spectrometer with discriminator window settings of 50 and 225 with an amplification factor of 60 per cent. Duplicate two minute counts were made. The presence of residual albumin I^{131} in the samples after alcohol precipitation did not alter the tritiated water counts with the discriminator setting that was utilized.

CALCULATIONS

From the activity of the indicator in each arterial sample a ratio of the concentration in the sample and the amount of indicator injected was derived. Thus the points used to construct the time-concentration curve of the indicator were independent of the absolute amount of indicator injected and the dilution curve of the Gamma emiting indicator could be compared directly with the dilution curve of the Beta emiting indicator. A Univac 418 digital computer was used to convert actual counts to concentration ratio points; to plot the time-concentration curve of the indicator; to obtain the slope of the exponential decay of the curve by a least-squares method; and to correct for recirculation by extrapolation of the

downslope. From these data the intravascular flow was calculated from the relationship:

$$F = \frac{I}{\int_0^\infty c(t)\,dt} \qquad (1)$$

where F = intravascular flow

I = total amount of indicator injected

and $\int_0^\infty c(t)\,dt$ = area under the curve.

The volume into which the indicator was distributed between injection and sampling sites was determined for each of the indicators by the mean transit time and calculation:

$$MTT = \frac{\int_0^\infty c(t)t\,dt}{\int_0^\infty c(t)\,dt} \qquad (2)$$

where MTT = mean transit time

$\int_0^\infty c(t)t\,dt$ = sum of the products of c(t) x t of the plotted curve.

The product of equations 1 and 2 is the mean transit time volume:

$$Q_{MTT} = F \times MTT \qquad (3)$$

The theoretical basis for the calculation of volumes by this method has been reviewed by Zierler (4).

The pulmonary extravascular water volume (Q_{pevw}) or perivascular distribution volume was obtained by subtracting Q_{MTT} calculated for Albumin I^{131} from Q_{MTT} calculated for tritiated water as follows:

$$Q_{pevw} = Q_{MTT_{(THO)}} - Q_{MTT_{(ALB)}} \qquad (4)$$

RESULTS

RECOVERY OF TRITIATED WATER

The calculation of distribution volumes from indicator dilution curves depends upon the assumption that none of the indicator is lost between the site of injection and the site of sampling. The ratio of the area of the tritiated water curve and the albumin curve expressed as percentage is a function of the recovery of tritiated water and other inaccuracies of a method that depends on pipetting several times, isotope counting and extrapolation of data points. The recovery of tritiated water averaged 95%. If the tritiated water/albumin ratio was less than 78% the measurement was excluded from further analysis.

RELIABILITY OF THE MEASUREMENT

From the series of 16 experiments, 35 duplicate sets of measurements of Q_{pevw} were available. Each measurement of a duplicate set was taken under essentially the same conditions and the measurements were separated in time by approximately 20 minutes. Sets were taken at sea level and after periods of exposure to altitude for 24, 48, and 72 hours. The test-retest reliability coefficient of the paired determinations of Q_{pevw} was 0.84 which indicates that the method of this volume is reproducible within the range of conditions encountered in this study. It was not possible to control respiration during the experiments. In human subjects it has been demonstrated that changes in Q_{pevw} approaching 30% may occur when a measurement is made with breath held in the end-tidal position and when the breath is held in inspiration (3). This variation is not seen in anesthetized animals when respiration is controlled or in humans who have voluntarily controlled inspiration (3).

CHANGES IN PULMONARY EXTRAVASCULAR WATER VOLUME WITH EXPOSURE TO SIMULATED HIGH ALTITUDE

Data from 16 experiments are summarized in Table 1. The number of successful experiments decreased with the duration of the experiment due to technical problems such as clotting of the indwelling catheters, poor flow from the sampling catheter, altitude chamber pump dysfunction, etc. Data from 15 experiments were technically satisfactory after exposure of 24 hours; 13 experiments after 48 hours; and 7 experiments after 72 hours exposure. Ten of the 11 animals had an increase in Q_{pevw} during altitude exposure although in one animal the change was trivial. After 24 hours exposure the mean increase in Q_{pevw} was 42.7 ml ($p < .025$); after 48 hours 79.3 ml ($p < .005$); and after 72 hours, 29.3 ml (N.S.). There was no significant difference in Q_{pevw} for the same duration of exposure at 12,000 feet and 16,000 feet.

Table II depicts sequential changes in mean Q_{pevw}. In the table each line is a subset of data from animals for which there were complete consecutive measurements at 24, 48, and 72 hours rexpectively. For example, if an animal had technically satisfactory Q_{pevw} determinations at 24 hours and 72 hours but unsatisfactory measurements at 48 hours, data from this animal do not appear in lines 2 or 3. This form of analysis showed highly significant increases in Q_{pevw} across 24 and 48 hours and an insignificant change from the control mean across 72 hours. It would appear from the table that the animals having complete measurements at 72 hours had a different sequence of Q_{pevw} expansion than animals with complete data at 48 hours.

Table I

Change in Q_{pevw} with Exposure to Altitude

	Hours at Altitude		
	24 Hours	48 Hours	72 Hours
Number	15	13	7
Control Volume (Mean ± SD)	242 ± 94	246 ± 108	246 ± 101
Volume After Exposure (Mean ± SD)	284 ± 82	325 ± 118	275 ± 97
F-Ratio	7 . 01	13 . 3	4 . 08
Significance of Difference	$p < .025$	$p < .005$	$p < .10$

Table II

Sequential Changes in Mean Q_{Pevw} with Length of Exposure

Subset	Number*	Control Volume	Length of Exposure 24 Hours	48 Hours	72 Hours	F-Ratio	p Value
1.	15	242	284			7.01	< .025
2.	11	229	279	313		7.27	< .005
3.	6	218	271	253	252	1.56	N.S.

*See text for explanation of differences between Table I and Table II

DISCUSSION

The pathogenesis of high altitude pulmonary edema has not been elucidated despite clinical (5,6), hemodynamic (5,7,8), and post mortem (9,10) studies in a large number of patients with this condition. Research has been impeded by the fact that high altitude pulmonary edema appears sporadically in human subjects and by the logistics of conducting complex cardiorespiratory measurements at high terrestial elevations. Pulmonary edema has been produced in several mammalian species by forcing the animal to exercise while breathing an atmosphere containing 3-10% oxygen (11,12) However, the response to high altitude varies widely among species and a satisfactory experimental model of human high altitude pulmonary edema has not been available.

Pulmonary edema can be produced in experimental animals by overloading the circulation with fluid or by administering alloxan which presumably alters capillary permeability (1). With hemodynamic overload, pulmonary capillary pressure and volume are increased while in alloxan edema both items are decreased (1). In humans studied during high altitude pulmonary edema left atrial pressure and the pulmonary capillary pressure measured indirectly by wedging the catheter in the pulmonary artery are normal or low. In six subjects taken to 14,246 feet, Weiskopf and Severinghaus found a fall in the pulmonary capillary volume measured by the carbon monoxide diffusing method after 52-56 hours of exposure. The subjects did not have the clinical signs of pulmonary edema, however (13). Severinghaus has proposed that hypoxic pulmonary hypertension might produce pulmonary edema by transarterial leakage (11). To support this theory, 12-35 micrometer polystyrene microspheres were introduced as emboli into the pulmonary arterial bed of rats. Pulmonary hypertension was found to be associated with perivascular edema and occasionally perivascular hemorrhage (11).

In pulmonary edema induced by either alloxan or increased hydrostatic pressure the earliest manifestation of edema is the appearance of fluid in the interstitial space (1) which can be measured by indicator dilution techniques or assessed by rapid freezing methods (14). Electron microscopy has revealed that the pulmonary interstitial space contains collagen fibrils and in pulmonary edema due to increased hydrostatic pressure the space expands greatly without disruption suggesting that it serves as a reservoir to collect excess fluid (2). We have hypothesized that the transcapillary movement of fluid into the interstitial compartment increases with exposure to high altitude. In some individuals the interstitial compartment is saturated and the capacity for removal of fluid by the lymphatic system is exceeded,

fluid accumulates in the alveoli, and overt pulmonary edema supervenes. In others, interstitial edema may occur in a subclinical form, while in other individuals the lymphatic flow may be adequate to remove the excess fluid and there is no accumulation. Our data support the major premise of this hypothesis. Ten of the 11 animals had an increase in Q_{pevw} during altitude exposure and the mean change from the control was highly significant after 24 and 48 hours of exposure. The peak increase of Q_{pevw} occurred after 48 hours of exposure which is consistent with the peak incidence of high altitude pulmonary edema in unacclimatized individuals taken to altitude.

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