The lung at high altitude: bronchoalveolar lavage in acute mountain sickness and pulmonary edema

ROBERT B. SCHOENE, ERIK R. SWENSON, CHRISTOPHER J. PIZZO, PETER H. HACKETT, ROBERT C. ROACH, WILLIAM J. MILLS, JR., WILLIAM R. HENDERSON, JR., AND THOMAS R. MARTIN

Divisions of Respiratory and Critical Care Medicine and Allergy and Infectious Diseases, Department of Medicine, University of Washington 98104; Medical Research Service of the Seattle Veterans Administration Medical Center, Seattle, Washington 98108; Department of Pathology, St. Anthony's Hospital, Denver, Colorado 80204; and Denali Medical Research Project, Section of Environmental Medicine, Center for High Latitude Research, University of Alaska, Anchorage, Alaska 99501

SCHOENE, ROBERT B., ERIK R. SWENSON, CHRISTOPHER J. PIZZO, PETER H. HACKETT, ROBERT C. ROACH, WILLIAM J. MILLS, JR., WILLIAM R. HENDERSON, JR., AND THOMAS R. MARTIN. *The lung at high altitude: bronchoalveolar lavage in acute mountain sickness and pulmonary edema.* J. Appl. Physiol. 64(6): 2605–2613, 1988.—High-altitude pulmonary edema (HAPE), a severe form of altitude illness that can occur in young healthy individuals, is a noncardiogenic form of edema that is associated with high concentrations of proteins and cells in bronchoalveolar lavage (BAL) fluid (Schoene et al., *J. Am. Med. Assoc.* 256: 63–69, 1986). We hypothesized that acute mountain sickness (AMS) in which gas exchange is impaired to a milder degree is a precursor to HAPE. We therefore performed BAL with 0.89% NaCl by fiberoptic bronchoscopy in eight subjects at 4,400 m (barometric pressure = 440 Torr) on Mt. McKinley to evaluate the cellular and biochemical responses of the lung at high altitude. The subjects included one healthy control (arterial O_2 saturation = 83%), three climbers with HAPE (mean arterial O_2 saturation = 55.0 ± 5.0%), and four with AMS (arterial O_2 saturation = 70.0 ± 2.4%). Cell counts and differentials were done immediately on the BAL fluid, and the remainder was frozen for protein and biochemical analysis to be performed later. The results of this and of the earlier study mentioned above showed that the total leukocyte count ($\times 10^5$/ml) in BAL fluid was 3.5 ± 2.0 for HAPE, 0.9 ± 4.0 for AMS, and 0.7 ± 0.6 for controls, with predominantly alveolar macrophages in HAPE. The total protein concentration (mg/dl) was 616.0 ± 3.3 for HAPE, 10.4 ± 8.3 for AMS, and 12.0 ± 3.4 for controls, with both large- (immunoglobulin M) and small- (albumin) molecular-weight proteins present in HAPE. There was evidence of complement activation (C5a) and release of thromboxane B_2 and leukotriene B_4 in HAPE but not in controls or AMS. Despite gas exchange impairment in AMS, the BAL fluid showed no evidence of abnormal protein or cell concentrations.

hypoxia; noncardiogenic edema; airflow obstruction; interstitial edema

HIGH-ALTITUDE PULMONARY EDEMA (HAPE) is a noncardiogenic form of pulmonary edema that occurs in ~1–2% of healthy individuals ascending above 4,000 m. Using bronchoalveolar lavage (BAL), we recently showed that climbers with HAPE at 4,400 m on Mt. McKinley had alveolar fluid with high concentrations of proteins and cells, primarily alveolar macrophages (20). The high protein concentration suggested that HAPE involved a major change in permeability of the alveolar capillary membrane, but the cell profiles suggested that the physiological and biochemical mechanisms of HAPE are different from other forms of permeability edema that may be mediated in part by polymorphonuclear leukocytes (24). The cause of the edema and its time course at high altitude are still unknown.

Acute mountain sickness (AMS) is a more common form of high-altitude illness that can be associated with HAPE and is characterized by impaired gas exchange (21). This milder form of dysfunction in the lung may reflect interstitial edema that may or may not progress to HAPE or may involve airflow obstruction that may predispose climbers to developing HAPE. We hypothesized that alveolar fluid in patients with AMS would have lower protein concentrations and a less intense cellular response than in patients with HAPE.

The purpose of this study, therefore, was to compare the cellular and biochemical content of alveolar lavage fluid at high altitude in normal healthy subjects and in climbers with AMS and HAPE.

METHODS

Research facility. All studies were performed on Mt. McKinley in a research facility at ~4,400 m (mean barometric pressure = 440 Torr) established by the Denali Medical Research Project. The primary purpose of this facility is to study high-altitude illness and, if necessary, to act as a medical facility for those who are ill enough not to be able to descend under their or their climbing party's own power. The research shelters were heated Hansen Weatherports (Gunnison, CO) located near the West Buttress of Mt. McKinley, where 400–600 climbers travel each spring to climb the mountain. The huts had telephone contact with both Talkeetna and Anchorage, AK, for medical air rescue assistance if necessary. Electrical power was supplied by solar panels and gasoline generators. The investigators reached the labo-

ratories in 3–5 days by skiing from an airstrip on the Kahiltna glacier at 2,200 m outside Denali National Park. The laboratory was supplied with standard resuscitation equipment.

Subjects (*Table 1*). The seven subjects all were climbers (mean age 33.3 ± 8.2 yr) with a spectrum of clinical altitude illness. Two were female. All volunteered for the study and gave informed consent as approval by the University of Washington Human Subjects Review Committee. Three of the subjects (ages 24, 39, and 26 yr) had overt signs and symptoms of high-altitude pulmonary edema, with fatigue, resting tachypnea and dyspnea, tachycardia, cyanosis, cough, arterial hemoglobin-O_2 desaturation (mean 55.0 ± 5.0% vs. normal at 4,400 m of ~83%), and rales on chest auscultation that were initially in the right middle lobe and progressed to other lung regions during the course of the observation. Each of these subjects had ascended rapidly from the airstrip at 2,200 m to the 4,400-m level in 2–3 days.

Four subjects (mean age 35.8 ± 9.5 yr) had mild-to-moderate symptoms of AMS, with headache, lethargy, decreased exercise performance, and difficulty in sleeping. None had severe AMS, which we defined as substantial functional impairment from more extreme symptomatology without overt signs of cerebral or pulmonary edema. All were ambulatory and capable of at least modest function around their respective camps and came to the research facility voluntarily for evaluation. All had relative arterial O_2 hemoglobin desaturation (mean 70 ± 2.4%) and the three subjects whose arterial blood was sampled had widened alveolar-arterial O_2 differences of 13, 15, and 18 Torr. Two had faint localized rales on chest auscultation in the right middle lobe area. None had cough, sputum, or resting dyspnea, although each had a resting tachycardia (>100 beats/min) and exercise dyspnea. One healthy female subject (age 34 yr) who had been living for ~2 wk at 4,400 m was a control subject. Her data were similar to those of three previous controls (20), and for comparison with the other groups, her results were combined with the others.

A primary concern of the investigative team was the health and safety of these climbers who volunteered for the studies and entrusted their medical care to the investigators. In no case was medical treatment (essentially rest and O_2 therapy) delayed. All subjects improved their clinical status and O_2 saturation with supplemental O_2 before and during the bronchoscopy procedure.

Because this study was, in part, designed to confirm and extend the results of our earlier study (20), we included the data from three HAPE and three control subjects from that study in the tables and figures for comparison. The primary difference in the procedure between the two studies was that in the present study, the cell counts, staining, and cell differentials were done immediately after the lavage at high altitude to ensure optimal evaluation of the cellular content. In the first study, the lavage fluids were frozen at altitude, then thawed in the laboratory at sea level, which could have influenced the cell counts because of lysis of the cells. As it turned out, the results in both studies for the two techniques were similar.

Procedure. All subjects came to the laboratory voluntarily, and the procedure was explained to them. They were monitored with an electrocardiogram and an ear oximeter (Hewlett-Packard), and low-flow O_2 via nasal cannulas was delivered to maintain arterial O_2-hemoglobin saturation at or above 90%. Atropine sulfate (0.6 mg) and diazepam (5–10 mg) were given intravenously 15 min before the study. Lidocaine (2% solution) was sprayed in the oral pharynx and upper airways to provide local anesthesia. A flexible fiber-optic bronchoscope (Pentax, model FB-15H) was passed through an oral airway and wedged in a subsegment of the right middle lobe. Three 40-ml aliquots of sterile pyrogen-free 0.89% NaCl were instilled into the lung, and then each was removed by gentle suction. A mean of 45.1 ± 6.1% of the instilled fluid was retrieved. All subjects tolerated the procedure well and were observed by an investigator (RBS, PHH, or ERS) overnight while receiving low-flow O_2 therapy via nasal cannulas. Their safety before descent was ensured by the investigative team.

Lavage fluid preparation and analysis. The bronchoalveolar lavage fluid was treated immediately after the procedure in the following manner. The individual aliquots were filtered through 4 × 4 in. gauze pads moistened with 0.89% NaCl to remove mucus and other debris. Separate aliquots of 1.0 ml were frozen for later cellular analysis in Seattle, WA. Total cell counts were performed immediately on the unspun aliquots in a hemocytometer, and the remaining fluid was centrifuged for 10 min to sediment the cells. The supernatant fluid was withdrawn and stored frozen in an underground igloo

TABLE 1. *Characteristics of subjects grouped by clinical severity of illness*

Subj No.	Group	Age/Sex	Sa_{O_2}, %	Pa_{O_2}, Torr	Rales	Resting Tachycardia	Resting Dyspnea	Cough Sputum
09	Normal	34/F	82	–	–	–	–	–
10	AMS	49/M	70	–	+	+	–	–
12	AMS	30/M	73	–	–	+	–	–
13	AMS	28/M	71	–	–	+	–	–
14	AMS	36/F	67	47.0	+	+	–	–
07	HAPE	24/M	55	40.5	+++	+	+	+
08	HAPE	39/M	50	40.5	++	+	+	+
11	HAPE	26/M	59	–	+++	+	+	+

Normal, healthy; acute mountain sickness (AMS), clinical illness of headache, lethargy, insomnia, mild arterial O_2-hemoglobin desaturation, without evidence of overt pulmonary edema; and high altitude pulmonary edema (HAPE), clinical spectrum of dyspnea at rest, cough, tachycardia, severe arterial O_2-hemoglobin desaturation, and crackles on chest auscultation.

(~−10°C) for later biochemical analyses in Seattle. The cell pellets were resuspended in 1.0 ml of Gey's balanced salt solution (GIBCO, Grand Island, NY). Total cell counts were performed in a hemocytometer, and cell viability was measured with the use of trypan blue. Aliquots of the resuspended cells were air-dried on glass slides and stained with Diff-Quik (Scientific Products, McGraw Park, IL), and differential cell counts were done on 200 cells. A venous blood sample was drawn from each subject at the time of the lavage. Hemoglobin concentrations and cell counts were measured on heparinized samples, and serum was frozen for protein analysis in Seattle.

Lavage fluid proteins. The lavage fluids were transported on dry ice to Seattle by air, where they were stored at −70°C until analyzed (TRM). The lavage fluids were thawed and concentrated approximately fivefold by positive-pressure filtration at 4°C under N_2 by use of a membrane with a 5,000-mol wt limit (YM-5, Amicon, Danvers, MA). The recovery of total serum proteins after concentration exceeds 85% with this method, as determined from separate control experiments.

The total proteins in the lavage fluid were analyzed by modification of the Lowry method, adapted to microtiter plates (12). Specific protein species were analyzed by radialimmunodiffusion with the use of commercially available plates (Calbiochem, LaJolla, CA). The protein species were chosen to reflect a range of molecular weights (Ig M = 900,000, Ig G = 150,000, and albumin = 67,000). Each immunodiffusion plate was standardized with the use of known concentrations of the specific protein measured. Complement (C5a) was measured in the samples by radioimmunoassay as described (15).

Eicosanoid radioimmunoassays. Thromboxane B_2 (TxB_2, the stable hydrolysis product of TxA_2), 6-keto-prostaglandin $F_{1\alpha}$ [6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of prostacyclin (PGI_2)], and leukotriene (LT) B_4, and LTC_4 were assayed in unextracted lavage fluid by radioimmunoassay. Each assay was performed in duplicate according to standard protocols (19). Unlabeled TxB_2 and 6-keto-$PGF_{1\alpha}$ standards were kindly provided by D. McCarter (Upjohn, Kalamazoo, MI), and unlabeled LTB_4 and LTC_4 standards were the generous gifts of Dr. J. Rokach (Merck-Frosst Laboratories, Pointe-Claire/Dorval, Quebec). Labeled tracers ([^{3}H]TxB_2, [^{3}H]6-keto-$PGF_{1\alpha}$, and [^{3}H]LTC_4) were obtained from New England Nuclear. The anti-TxB_2 and anti-6-keto-$PGF_{1\alpha}$ were prepared in our laboratory by first conjugation of TxB_2 and 6-keto-$PGF_{1\alpha}$, respectively, to thyroglobulin by the mixed-anhydride method, followed by immunization of the rabbits. The TxB_2 antiserum at a dilution of 1:100,000 had a sensitivity of ~1 pg/0.1 ml sample, and the following cross-reactivities at B/B_0, 50%: PGD_2, 0.53%; $PGF_{2\alpha}$, 0.2%; $PGF_{1\alpha}$, 0.02%; and PGE_2, 6-keto-$PGF_{1\alpha}$, and 6-keto-PGE_1, each <0.02%. The 6-keto-$PGF_{1\alpha}$ antisera at a dilution of 1:3,000 had a sensitivity of ~10 pg/0.1 ml sample and the following cross-reactivities at B/B_0 50%: 6-keto-PGE_1, 2.5%; $PGF_{1\alpha}$, 1.43%; $PGF_{2\alpha}$, 0.77%; PGD_2, 0.42%; and TxB_2, PGE_1, and PGE_2 each <0.11%. Rabbit anti-LTB_4 and anti-LTC_4 antisera were the kind gifts of Drs. Robert W. Egan and John L. Humes (Merck Institute for Therapeutic Research, Rahway, NJ). The LTB_4 and LTC_4 antisera, at a dilution of 1:4,500 and 1:60,000, respectively, each had a sensitivity of ~10 pg/0.1 ml sample; the cross-reactivities of these antisera have been reported previously (19).

Statistical analysis. The data were grouped for subjects with HAPE, AMS, and the healthy controls. Justification for combining the data from 1983 and 1985 was based on the fact that the means from the two studies in each group were not statistically different. Specifically, they were less than one standard deviation away from each other in each case. Initial comparisons were made by use of one-way analysis of variance. Secondary comparisons were made with the use of Student's t test. A P value < 0.05 was considered significant.

RESULTS

All subjects tolerated the lavage procedure well and recovered fully from their clinical illness. The three subjects with HAPE descended to ~2,200–3,000 m to recover, and two of the subjects climbed to the summit within 7–14 days. The four subjects with AMS recovered with further acclimatization at 4,400 m and then went to the summit.

Lavage cells. The cell types in lavage fluid are shown in Fig. 1. The alveolar macrophages from the subjects with HAPE tended to have more abundant foamy cytoplasm and larger nuclei with more prominent nucleoli, and the HAPE fluids contained more erythrocytes than control fluids. Occasional erythrophagocytic macrophages were also noted in HAPE fluids.

The results of the lavage fluid cell counts and differentials are shown in Table 2 and are grouped by the year of study and the clinical illness. Statistical analysis of the cell counts, both by percent and absolute numbers, yielded identical results. Subjects with HAPE had the greatest leukocyte concentrations in the lavage fluid. The leukocyte concentrations in these subjects were similar in the subjects studied in 1983 compared with those studied in 1985. The lavage cell concentrations in the patients with AMS and in the normal subjects were similar to those of normal volunteers studied at sea level (14). In the subjects with HAPE, ~90% of the cells were viable. This is similar to the viability of lavage cells at sea level (14). The reason for the lower cell viability in the normal subject (*subject 09*) is not clear. Subjects with HAPE tended to have higher percentages of neutrophils in lavage fluid than those with AMS, although there was variability (range 4.0–44.0%). Three of the subjects with HAPE had >10% polymorphonuclear leukocytes (PMN) in lavage fluid, a value that is considered abnormal in most laboratories (2, 14). One subject (*subject 07*) had 44% PMN, and on bronchoscopy there was evidence of frothy fluid in the lower airways. Historically, he had had 2–3 days of dry cough, before he developed HAPE. None of the stains showed evidence of bacteria. The differences in the percentages of neutrophils between the groups were not statistically significant because of the variability in the HAPE subjects.

Lavage proteins. The protein concentrations in the lavage fluid are shown in Table 3 grouped by year of

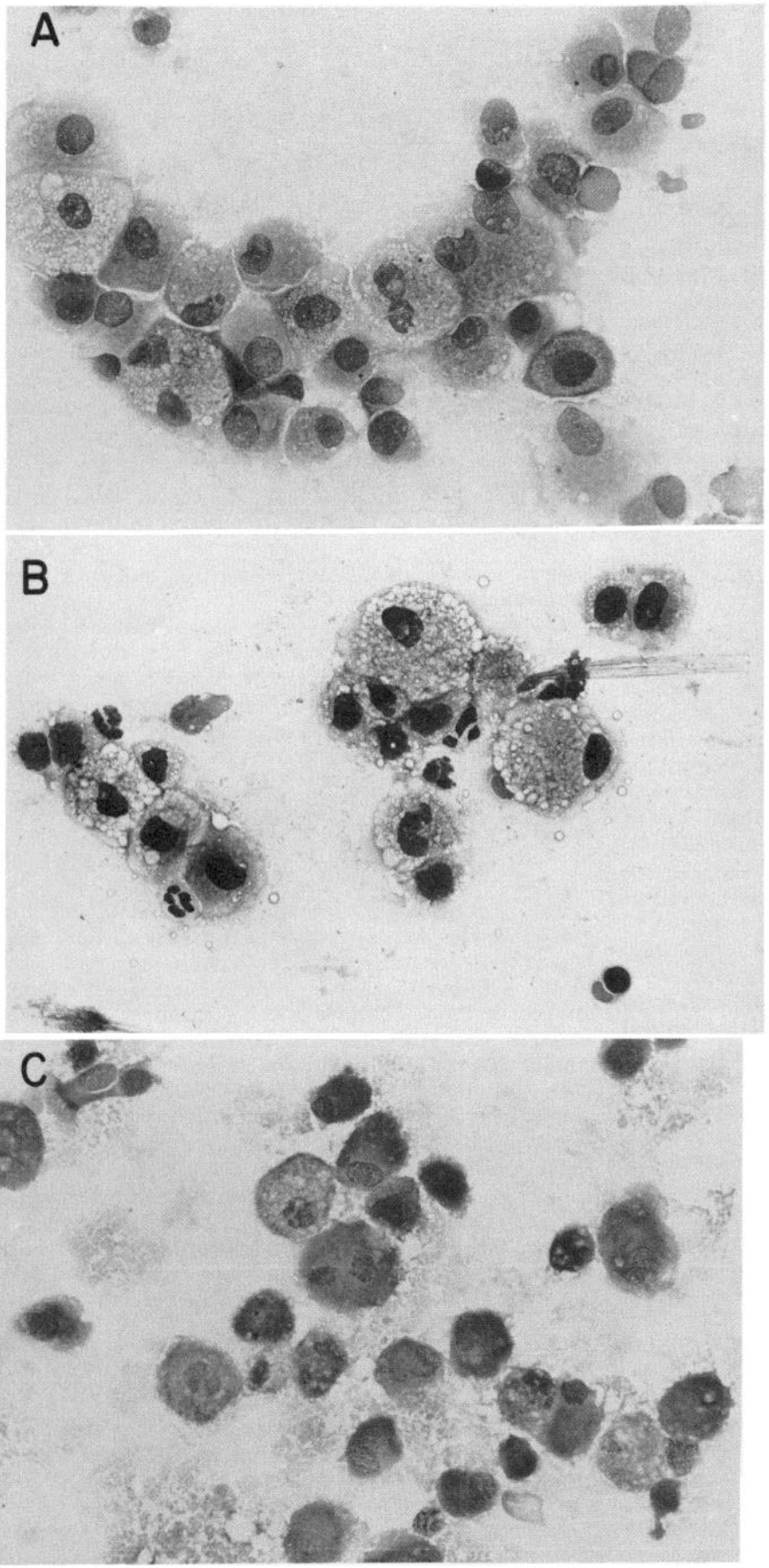

FIG. 1. Bronchoalveolar lavage cells from a normal subject at high altitude (*A, subject 9*), a subject with clinical high-altitude mountain sickness (HAPE) (*B, subject 11*), and a subject with acute mountain sickness without clinical HAPE (*C, subject 17*). Alveolar macrophages from subject with HAPE (*B*) tended to be larger and more vacuolated than those of normal subject, and some neutrophils also can be seen. Alveolar macrophages of subject with acute mountain sickness appear normal (*C*) and neutrophils are not seen.

TABLE 2. *Lavage cell counts and differentials grouped by clinical severity of illness*

Subj No.	Year	Group	Total Leukocytes, $\times 10^6$/ml	Viability, %	Differential, %		
					AM	PMN	Lym
02	1983	Normal	0.25		96.0	2.0	2.0
03	1983	Normal	0.35		86.0	5.0	9.0
05	1983	Normal	0.70		96.0	2.0	2.0
09	1985	Normal	1.63	76.5	97.0	2.0	1.0
Mean ± SD			0.73±0.63		93.8±5.2	2.8±1.5	3.5±3.7
10	1985	AMS	0.70	91	97.0	1.5	1.5
12	1985	AMS	0.80		96.0	0.5	3.5
13	1985	AMS	0.50		89.5	3.5	7.0
14	1985	AMS	1.45		92.7	4.0	3.3
Mean ± SD			0.86±0.41		93.8±3.4	2.4±1.7	3.8±2.3
01	1983	HAPE	1.35		85	13	2
04	1983	HAPE	6.85		78	7	15
06	1983	HAPE	2.80		73	4	23
07	1985	HAPE	2.45	92.5	38.0	44.0	18.0
08	1985	HAPE	2.73	99.5	94.0	4.3	1.7
11	1985	HAPE	4.55	89.0	70.3	27.8	2.0
Mean ± SD			3.46±1.96*	93.7±5.3	67.4±28.1	25.4±20.0	7.2±9.3

AM, alveolar macrophages; PMN, polymorphonuclear leukocytes; Lym, lymphocytes. See legend of Table 1 for definitions of other abbreviations and details of groups. * $P < 0.025$ compared with AMS and with normal subjects.

TABLE 3. *Lavage protein and eicosanoid concentrations grouped by clinical severity of illness*

Subj No.	Year	Group	Total Protein, mg/dl	Albumin, mg/dl	IgG, mg/dl	IgM, mg/dl	C5a, pg/ml	TxB_2, pg/ml	6-keto-$PGF_{1\alpha}$, pg/ml	LTB_4, pg/ml	LTC_4, pg/ml
02	1983	Normal	8.0	4.7	0.4	ND		50	980	ND	ND
03	1983	Normal	16.0	7.6	0.7	ND		53	980	ND	ND
05	1983	Normal	11.0	6.8	0.4	ND		48	890	ND	ND
09	1985	Normal	13.0	7.8	1.9	ND	25.6	39	900	ND	ND
Mean ± SD			12.0±3.4	6.7±1.4	0.9±0.7		25.6	42.5±6.0	937.5±49.2		
10	1985	AMS	9.0	4.0	1.5	ND	24.0	40	790	ND	ND
12	1985	AMS	4.9	3.7	0.8	ND	20.8	37	740	ND	ND
13	1985	AMS	5.2	3.4		ND	30.4	39	1,030	ND	ND
14	1985	AMS	22.4	10.0	4.2	ND	24.0	36	1,130	ND	ND
Mean ± SD			10.4±8.3	5.3±2.7	2.2±1.5		24.8±4.0	38.0±1.8	922.5±187.5		
01	1983	HAPE	507	330	63	4.3		540	1,700	520	ND
04	1983	HAPE	1,136	660	152	7.0		500	850	1,350	ND
06	1983	HAPE	376	180	42	4.6		84	1,220	870	ND
07	1985	HAPE	840	630	153	13.8	52.0	440	840	1,050	ND
08	1985	HAPE	610	280	108	5.0	44.0	390	1,250	300	ND
11	1985	HAPE	227	140	55	8.8	36.0	100	600	770	ND
Mean ± SD			616.0±329.1†	370.0±223.8†	95.5±49.4†	7.3±3.6	44.0±8.0†	342.3±200.6*	1,076.7±393.4	810.0±373.8	

IgG, IgM, immunoglobins G and M; TxB_2, thromboxane B_2; LTB_4, LTC_4, leukotrienes B_4 and C_4. See legend of Table 1 for definitions of other abbreviations and details of groups. * $P < 0.02$, † $P < 0.01$ compared with AMS and with normal subjects (C5a is compared only with AMS). ND, not detectable.

study and clinical illness. The volume of retrieved fluid was nearly identical in all subjects, making it unlikely that variations in fluid recovery affected the protein measurements. The subjects with severe HAPE had marked increases in the lavage total protein concentration. The protein concentrations in subjects studied in 1983 (*subjects 01, 04,* and *06*) were in the same range as those of subjects studied in 1985. The lavage protein concentrations of subjects with AMS did not differ from those of the normal subjects who were studied at altitude, and each of these latter groups had protein concentrations that were within the normal range for normal subjects studied at sea level (upper limit of normal 25.0 mg/dl; $n = 21$; Ref. 14).

The subjects with HAPE also had corresponding increases in the concentrations of albumin and IgG in lavage fluid, but the ratio of albumin concentration to total protein concentration was similar in all groups (HAPE = 60.1%, AMS = 55.8%, and normal = 55.8%). The relative concentration of IgG to albumin was slightly

higher in the subjects with HAPE and AMS compared with normal subjects (HAPE = 15.5%, AMS = 21.1%, and normal = 7.5%). IgM was easily detectable in all of the subjects with HAPE but was not detectable by the radial immunodiffusion technique in either the subjects with AMS or the normal subjects, even when the lavage fluids were concentrated five-fold by positive-pressure filtration. The concentration of C5a, measured in the subjects studied in 1985 was greatest in the subjects with HAPE ($P < 0.01$). The concentrations of C5a in lavage fluids of subjects with AMS and the normal subjects were near the lower limit of detection in the assay (~20.0 pg/ml).

The relationships between lavage and serum proteins for the subjects studied in 1985, for whom both serum and lavage specimens were available are shown in Table 4. We measured three different protein species: albumin, IgG, and IgM. These proteins were chosen because they represent a range of molecular weights and because they are not synthesized locally in the air space of the lung in individuals without preexisting lung disease. Therefore alterations in the relative distributions of these proteins should provide evidence concerning the permeability of the lung to different sizes of proteins. In the subjects with HAPE, the lavage-to-serum ratios for total protein, albumin, and IgG were much higher than in the subjects with AMS. These latter subjects had ratios that were similar to those seen in the healthy individual. The lavage-to-serum ratios for IgM were less than those for albumin and IgG in the subjects with severe HAPE, suggesting that some protein selectivity remained in the lungs of these individuals. In contrast, no IgM was detectable in the lavages of subjects with AMS and the normal individuals, indicating that the normal barrier of this protein was intact in these subjects.

Cyclooxygenase (TxB_2 and 6-keto-$PGF_{1\alpha}$) and 5-lipoxygenase (LTB_4 and LTC_4) products of arachidonic acid metabolism were assayed by radioimmunoassay in the lavage fluids. Significantly elevated levels of TxB_2 and LTB_4 were found in the lavage samples of HAPE patients compared with either normal controls or patients with AMS (Table 3). Comparable amounts of 6-keto-$PGF_{1\alpha}$, the stable breakdown product of PGI_2, were found in the lavage fluids of the three subject groups. LTC_4 was not observed in lavage fluids from the HAPE, AMS, or normal individuals (Table 3).

DISCUSSION

The results of this study confirm and extend our earlier findings that bronchoalveolar lavage fluid from patients with high-altitude pulmonary edema contains increases in a broad spectrum of proteins and increased cell concentrations with a predominance of alveolar macrophages. Furthermore the lavage fluid contains elevated levels of several potent chemical mediators (LTB_4, C5a, and thromboxane) that have the potential to recruit cells to the lung (15, 22) and cause vasoconstriction (5), respectively. In contrast, the findings in bronchoalveolar lavage fluid of hypoxic patients with acute mountain sickness were not substantially different with respect to proteins and cells from lavage fluids of healthy subjects at altitude or at sea level. These latter findings disproved one of our initial hypotheses, but, importantly, they suggest that the gas exchange abnormalities in AMS may be secondary to areas of low ventilation-perfusion match from airway narrowing and/or interstitial edema. Airway hyperreactivity is another possibility for which we have no data.

Our prior study demonstrated that climbers with HAPE had high concentrations of protein in BAL fluid, suggesting an increase in permeability of the alveolar membrane, whereas healthy controls had normal lavage fluid values (20). A shortcoming of that study was that no subjects with mild symptoms or mild arterial O_2-hemoglobin desaturation were included, so no insight into this intermediate group was gained. The present study was designed to include subjects with AMS as well as HAPE to extend our previous findings and to characterize more completely the range of cellular and biochemical findings in lung lavage fluid in humans at high altitude.

Additionally, the present study was designed to improve the evaluation of cellular content in the lavage fluid. The earlier study (20) depended on freezing the

TABLE 4. *Lavage-to-serum ratios for selected proteins in subjects studied in 1985*

Subj No.	Group	Total Protein	Albumin	IgG	IgM
07	HAPE	16.41	26.81	14.81	1.21
08	HAPE	12.10	8.51	12.53	0.70
11	HAPE	4.00	4.68	5.34	0.74
Mean ± SD		10.84±5.14*	13.33±9.66	10.89±4.04*	0.89±0.23†
10	AMS	0.16	0.11	0.09	
12	AMS	0.09	0.14	0.07	
13	AMS	0.09	0.16		
14	AMS	0.35	0.34	0.31	
Mean ± SD		0.17±0.11	0.19±0.09	0.16±0.11	
09	Normal	0.21	0.24	0.12	

Values are calculated as 100 × (lavage concentration/serum concentration), where lavage and serum concentrations are in mg/100 ml. Molecular weights of specific proteins are albumin = 67,000, IgG = 150,000, and IgM = 900,000. IgG, IgM, immunoglobins G and M. See legend of Table 1 for definitions of abbreviations and details of groups. * $P < 0.02$ vs. AMS. † $P < 0.02$ vs. HAPE IgG ratio.

fluid and doing cell counts and differentials at a later date on thawed specimens at sea level. This delay and freeze-thaw process could have led to cell lysis and destruction. In the present study the cell counts and differentials were done at altitude immediately after the lavage procedure. Additional aliquots of the fluid were frozen and evaluated at sea level by the same methods used in the earlier study. The cell counts generally were in agreement, but the cellular morphology was excellent in the freshly prepared specimens and poor in the frozen ones.

The observation that subjects with AMS had virtually normal lavage protein and cell values suggests that although gas exchange was impaired, the process that alters gas exchange does not alter the concentrations of proteins and cells in the air space, at least in the segment of lung that we lavaged. Considerable evidence suggests that the early changes in the lung at altitude that are detrimental to gas exchange and mechanics may reflect interstitial edema, which may progress to overt alveolar edema, i.e., HAPE (1, 3, 9–11). One possible mechanism of interstitial edema formation is that the shear forces associated with increased pulmonary vascular pressures might injure the vascular endothelium, promoting a leak of fluid from the vascular space to the interstitium of the lung (23). This possibility gains support from two types of clinical observations: *1*) the association of HAPE with congenital unilateral absence of the pulmonary artery (4), a situation in which high intravascular mechanical shear forces could exist at altitude; and *2*) the observation that subjects with HAPE have inordinately high pulmonary arterial pressure during illness and exaggerated pulmonary vascular responses to hypoxia after recovery (8). Although this finding has not been consistent in all studies, such brisk hypoxic pulmonary vasoconstrictive responses and pulmonary hypertension could contribute to shear stresses in the pulmonary vasculature.

Our finding of high concentrations of thromboxane in lavage fluids of subjects with HAPE lends biochemical support to this hypothesis, since thromboxane is a potent mediator of pulmonary vasoconstriction (5). Other effects of thromboxane relevant to pulmonary inflammation are its induction of bronchial smooth muscle contraction and platelet and neutrophil aggregation (5). Thromboxane is a major product of activated platelets and is also generated by alveolar macrophages. It is possible that the increased numbers of alveolar macrophages contribute at least in part to the increased thromboxane levels in the lavage fluids.

In contrast, there was no evidence for increased production of PGI_2 in lung lavages of the HAPE patients compared with normal or AMS subjects. Many of the biological effects of PGI_2 on the lung [e.g., dilation of pulmonary vascular beds, prevention of intrapulmonary platelet aggregation, and relaxation of bronchial smooth muscle (5)] are opposite to TxA_2, and failure to have augmented PGI_2 release in HAPE patients may contribute to the observed pathophysiological changes observed. LTC_4, which like TxA_2 induces pulmonary vasospasm and airway smooth muscle contraction, was not found in any of the lavage fluids obtained in this study (detection limit of the radioimmunoassay for LTC_4 is ~10 pg/0.1 ml sample). Should LTC_4 have been produced in lung fluids of these subjects, oxidative degradation of LTC_4 to sulfoxide derivatives and diastereoisomers of LTB_4 before performance of the radioimmunoassays could have limited detection of LTC_4 in the lavage samples.

Our findings that subjects with AMS have normal lavage fluid cells and proteins suggest but do not prove that these subjects are at an earlier stage of an illness that begins with interstitial edema and gas exchange abnormalities and progresses to overt high-protein alveolar edema and clinical HAPE. Because we have not documented progression from normal to abnormal lavage findings in any patient; however, it remains possible that AMS and HAPE are not on a continuum and that subjects with AMS may not progress to HAPE.

Subjects with severe HAPE had a marked cellular response in the lavage fluid which was composed of increases both of alveolar macrophages and PMNs, whereas in the control and AMS subjects both the total cell numbers and the cell differentials were normal. Because of the large number of total cells in the HAPE subjects, the absolute numbers of both neutrophils and macrophages were increased, but the relatively normal percentage of neutrophils in the four of the six HAPE lavage fluids is distinctly different from patients with other types of diffuse lung injury such as ARDS (24, 25). These findings suggest that a major increase in membrane permeability in the lung can occur independently of the presence and activity of air-space neutrophils, although their role is not completely understood. The observation that the adult respiratory distress syndrome (ARDS) can occurr in neutropenic patients (17) further supports this conclusion. The remarkable observation in the subjects with HAPE is that this injury resolves very rapidly, even at altitude, and that the subjects can continue to climb at altitude immediately after the resolution of their illness. The role of the alveolar macrophages is also not completely understood. It is not clear whether they play a causative role in the lung leak or are merely a response to the injury.

The relatively low percentage of neutrophils in subjects with HAPE may be secondary to a complex interaction of factors that influence the migration of cells from the vasculature into the lung. Higher levels of complement (C5a) and LTB_4 were present in the alveolar fluid of subjects with HAPE but not in those with AMS. Both substances are mediators of neutrophil chemotaxis (22), and LTB_4 is the predominant neutrophil chemotactic factor produced by resident human alveolar macrophages (15). In the earlier HAPE study (20), evidence suggested the presence of a chemotactic inhibitor that could inhibit the migration of neutrophils into the lung. Recent data from Robbins et al. (18) show that ARDS is characterized by the accumulation in the lavage fluid of a functionally inactive chemotactic factor inactivator in lavage fluid. It is possible that the balance between chemoattractants (C5a and LTB_4) and a chemotactic inhibitor derived from the plasma determines the neutrophil response in the lung fluid.

One subject (*subject 07*) with HAPE had a high neu-

trophil content (44%) in his BAL fluid. His overt HAPE was preceded by 2–3 days of cough, but his subsequent clinical course, which included rapid resolution on descent and a successful ascent of the rigorous Cassin Ridge, was most compatible with HAPE and not a viral or bacterial pneumonitis. His high neutrophil concentration is troublesome and indicates that either HAPE involves at some stage an influx of neutrophils that was missed in the other subjects or that a nonspecific bronchitis which is common at high altitude may have predisposed him to or occurred concomitantly with HAPE. Although alveolar macrophages are the predominant cell in the BAL fluid of HAPE subjects, there is an increased total number of neutrophils as well as some mediators (LTB_4 and C5a or chemotoxin) in HAPE BAL. One other subject (11) had a high neutrophil concentration (28%) as well. To exclude one or both of these subjects who clearly had HAPE would not be justified. Therefore the role of the neutrophil in HAPE remains somewhat obscure.

Another remarkable finding of the study is that climbers with HAPE have very high concentrations of proteins in the alveolar spaces with a spectrum of molecular weights, whereas healthy climbers and climbers with mild oxygen-hemoglobin desaturation and symptoms of AMS had virtually normal proteins and cells in their alveolar fluid. Also of interest is the finding that the protein concentration in the alveolar fluid in HAPE is very high, even higher than values reported in patients with ARDS (6, 13). Since climbers with HAPE can quickly recover fully, these high protein concentrations in the alveolar fluid do not seem to predict the reversibility of the lung injury.

The high protein concentrations could be secondary to concentration of the proteins as liquid is cleared from the alveoli more rapidly than the proteins (16). It was difficult in the field setting to control the time when lavage was performed relative to the onset of symptoms. Certainly, it is conceivable that in three of the climbers with HAPE in whom lavage was performed 24–48 h after the onset of their overt clinical symptoms, alveolar clearance of fluid and subsequent concentration of protein could have taken place. The other three subjects with HAPE were studied at approximately 4, 12, and 20 h after the onset of their respiratory symptoms. There was no correlation between protein concentrations and time of lavage. Therefore it is unlikely that differential clearance of liquid and protein from the lungs is the major explanation for the high lavage protein concentrations that we observed.

It is conceivable that in subjects with AMS we could have obtained false negative results. This possibility could have occurred because of the patchy distribution of HAPE, and lavage may not have been directed at an involved area. We do know that the AMS patients had relative hypoxemia, and it is not known whether this is secondary to diffuse areas of low ventilation-perfusion mismatch or shunt or to one involved area. Radiographic confirmation of disease distribution was not available, but the former possibility is much more likely.

We did lavage the right middle lobe area in which the findings were uniform in the HAPE subjects. It is our experience that HAPE consistently starts with physical findings of crackles in this area, which later may spread diffusely. Also, radiographic evidence from other clinical studies suggest that HAPE occurs predominantly in the right middle lobe area. It is not clear why this occurs, but it may have something to do with less collateral ventilation in the right middle lobe and potentially higher pulmonary vascular pressure. Therefore, we feel that it is certainly possible that we have missed some areas in the lung of involvement of disease, but since the findings were consistent in both the HAPE as well as the AMS subjects, we think that the lavage data from the AMS patients are a valid reflection of the condition of the airspace at this stage of their disease.

In summary, subjects with high-altitude pulmonary edema have a type of lung leak characterized by markedly increased protein and cell concentrations in the air space and high levels of TxB_2, a mediator of pulmonary hypertension, and LTB_4, a potent chemotactic factor for leukocytes. Agents that modify thromboxane production or the pulmonary vascular response might prevent or reverse the process that leads to HAPE. Additionally, subjects with AMS and mild arterial O_2 hemoglobin desaturation have lung lavage fluid that does not differ from healthy controls. These findings suggest that the gas exchange abnormality in subjects with AMS does not involve the alveolar space.

We are greatly indebted to the climbing rangers of the US National Park Service; Drs. Gil Roberts and Frank Hollingshead, who acted as general physicians for the research station; Kimberlee Carter and Kim Leatham, camp managers; and Laurie Nichols for her excellent help in preparation of the manuscript.

This study was supported in part by National Heart, Lung, and Blood Institute (NHLBI) Clinical Investigator Grant HL-00906 (R. B. Schoene) and an American Lung Association Trudeau Scholar Award (R. B. Schoene); Special Center of Research Grant (project 02) HL-30542, and a National Institute of Allergy and Infectious Diseases Academic Award AI-00487 (W. R. Henderson); NHLBI Grant HL33247; and the Research Service of the Veterans Administration (T. R. Martin).

Received 6 April 1987; accepted in final form 8 January 1988.

REFERENCES

1. COATES, G., G. GRAY, A. MANSELL, C. NAHMIAS, A. POULES, J. SUTTON, AND C. WEBBER. Changes in lung volume, lung density, and distribution of ventilation during hypobaric decompression. *J. Appl Physiol.* 6: 752–753, 1979.
2. CRYSTAL, R. G., J. E. GADEK, V. J. FERRANS, J. D. FULMER, B. R. LINE, AND G. W. HUNNINGHAKE. Interstitial lung disease: current concepts of pathogenesis, staging, and therapy. *Am. J. Med.* 70: 542–568, 1981.
3. GROW, G. W., M. MCFADDEN, C. S. HOUSTON, AND A. C. BRYAN. Changes in single-breath nitrogen washout curve on exposure to 17,600 feet. *J. Appl. Physiol.* 39: 652–656, 1975.
4. HACKETT, P. H., C. E. CREAGH, R. F. GROVER, B. HONIGMAN, C. S. HOUSTON, J. T. REEVES, A. M. SOPHOCLES, AND M. VAN-HARDENBROEK. High altitude pulmonary edema in persons without the right pulmonary artery. *N. Engl. J. Med.* 302: 1070–1073, 1980.
5. HENDERSON, W. R. Eicosanoids and lung inflammation. *Am. Rev. Respir. Dis.* 135: 1176–1185, 1987.
6. HOLTER, J. F., J. E. WEILAND, E. R. PACHT, J. E. GADEK, AND W. B. DAVIS. Protein permeability in the adult respiratory distress syndrome: loss of size selectivity of the alveolar epithelium. *J. Clin. Invest.* 78: 1513–1522, 1986.

7. HSUEH, W., F. GONZALEZ-CRUSSI, AND E. HANNEMAN. Prostaglandin synthesis in the different phases of phagocytosis in lung macrophages. *Nature Lond.* 283: 80–82, 1980.
8. HULTGREN, H. N., R. F. GROVER, AND L. H. HARTLEY. Abnormal circulatory responses to high altitude in subjects with a previous history of high altitude pulmonary edema. *Circulation* 44: 759–770, 1981.
9. HYERS, T. M., C. H. SCOGGIN, D. H. WILL, R. F. GROVER, AND J. T. REEVES. Accentuated hypoxemia at high altitude in subjects susceptible to high-altitude pulmonary edema. *J. Appl. Physiol.* 46: 41–46, 1979.
10. JAEGER, J. J., J. T. SYLVESTER, A. CYMERMAN, J. J. BERBERICH, J. C. DENISTON, AND J. T. MAHER. Evidence for increased intrathoracic fluid volume in man at high altitude. *J. Appl. Physiol.* 47: 670–676, 1979.
11. KRONENBERG, R. S., P. SOFAR, J. LEE, F. WRIGHT, W. NOBLE, E. WAHRENBROCK, R. HICKEY, E. NEMOTO, AND J. W. SEVERINGHAUS. Pulmonary artery pressure and alveolar gas exchange in man during acclimatization to 12,470 feet. *J. Clin. Invest.* 50: 827–837, 1971.
12. LOOMIS, T. C., AND W. L. STAHL. A rapid flexible method for the biochemical assays using a microtiter plate reader and a microcomputer. Application for assays of protein, Na, K-ATPase and K-*p*-nitrophenylphosphatase. *Int. J. Biomed. Comput.* 18: 183–192, 1986.
13. MAGUIRE, W. W., R. G. SPRAGGUE, A. M. COHEN, AND C. G. COCHRAN. Studies on the pathogenesis of the adult respiratory distress syndrome. *J. Clin. Invest.* 69: 543–553, 1982.
14. MARTIN, T. R., G. RAGHU, R. J. MAUNDER, AND S. C. SPRINGMEYER. The effects of chronic bronchitis in chronic airflow obstruction on lung cell populations recovered by bronchoalveolar lavage. *Am. Rev. Respir. Dis.* 132: 254–260, 1985.
15. MARTIN, T. R., G. RAUGHI, T. L. MERRITT, AND W. R. HENDERSON. The relative contribution of leukotriene B_4 to the neutrophil chemotactic activity produced by the resident human alveolar macrophage. *J. Clin. Invest.* 80: 1114–1124, 1987.
16. MATTHAY, M. A., C. C. LANDOLT, AND N. C. STAUB. Differential liquid and protein clearance from the alveoli of anesthetized sheep. *J. Appl. Physiol.* 53: 96–104, 1982.
17. MAUNDER, R. J., R. C. JACKMAN, E. RIFF, R. K. ALBERT, AND S. C. SPRINGMEYER. Occurrence of the adult respiratory distress syndrome in neutropenic patients. *Am. Rev. Respir. Dis.* 133: 313–316, 1986.
18. ROBBINS, R. R., R. J. MAUNDER, G. GOSSMAN, T. KENDALL, L. D. HUDSON, AND S. RENNARD. Functional loss of chemotactic factor inactivator in the adult respiratory distress syndrome (Abstract). *Am. Rev. Respir. Dis.* 133: A277, 1986.
19. ROKACH, J., E. C. HAYES, Y. GIRARD, D. L. LOMBARDO, A. L. MAYCOCK, A. S. ROSENTHAL, R. N. YOUNG, R. ZAMBONI, AND H. J. ZWEERINK. The development of sensitive and specific radioimmunoassays for leukotrienes. *Prostaglandins Leukotrienes Med.* 13: 21–25, 1984.
20. SCHOENE, R. B., P. H. HACKETT, W. R. HENDERSON, E. H. SAGE, M. CHOW, R. C. ROACH, W. J. MILLS, JR., AND T. R. MARTIN. High-altitude pulmonary edema: characteristics of lung lavage fluid. *J. Am. Med. Assoc.* 256: 63–69, 1986.
21. SINGH, I., P. K. KHANNA, M. C. SRIVASTAVA, M. LAL, S. B. ROY, AND C. S. V. SUBRAMANYAM. Acute mountain sickness. *N. Engl. J. Med.* 280: 175–184, 1968.
22. SNYDERMAN, R., AND E. J. GOETZL. Molecular mechanisms of chemotaxis. *Science Wash. DC* 213: 830–837, 1981.
23. STAUB, N. C. Pulmonary edema: hypoxia and overperfusion. *N. Engl. J. Med.* 302: 1085–1087, 1980.
24. TATE, R. M., AND J. E. REPINE. Neutrophils in the adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* 128: 552–560, 1983.
25. WEILAND, J. E., W. B. DAVIS, J. F. HOLTER, J. R. MOHAMMED, P. M. DORINSKY, AND J. E. GADEK. Lung neutrophils in the adult respiratory distress syndrome: clinical and pathophysiological significance. *Am. Rev. Respir. Dis.* 133: 218–226, 1986.