Fatty Change of the Granular Pneumocyte

Abstract. *Fat vacuoles develop in the granular pneumocytes of guinea pigs exposed to severe hypoxia in low-pressure chambers. The osmiophilic lamellar bodies are apparently reduced in size and decreased in number. The fatty change of the granular pneumocyte may represent a metabolic alteration and interfere with the production of surfactant. This hypoxic lesion of the pneumocyte may be a significant factor in high-altitude pulmonary insufficiency.*

Normal granular pneumocytes contain, in addition to the usual cellular components, characteristic cytoplasmic granules called lamellar bodies, which Schultz believes are derived from mitochondria (*1*). These cells are believed to produce lung surfactant (*2, 3*). Abnormalities of the lamellar bodies have been observed in the granular pneumocytes of guinea pigs exposed to 15 percent carbon dioxide and are also associated with pulmonary edema, atelectasis, and hyaline membrane formation (*4*). This report describes cytoplasmic alterations of the granular pneumocyte due to severe hypoxia.

Thirty adult male Hartley-Dunkirk albino guinea pigs were divided into five groups of six animals each. Group 1 was kept at sea-level pressure, and group 2 was exposed, step by step, to an atmospheric pressure equivalent to an altitude of 4267 m. Decompression proceeded as follows: day 1 to 2438 m, day 2 to 3658 m, and day 3 to 4267 m; the animals were maintained at this altitude for 6 months or more. Group 3 was decompressed to 5486 m, kept at this altitude for 48 hours, and then returned to sea level for study. Group 4 was exposed to 5486 m gradually as follows: day 1 to 2800 m, day 2 to 3658 m, and day 3, and for at least 6 weeks thereafter, to 5486 m. Group 5 was also kept at 5486 m for 6 weeks and at sea level for 1 week, and then returned to 5486 m for a final 24-hour exposure. The steel chambers used for all experiments were opened twice weekly for cleaning and feeding; the recompression to sea-level pressure and subsequent return to high altitude each took 1 hour. Exhausted air from the chambers was analyzed immediately before each opening and contained 20 to 21 percent oxygen and 0.20 to 0.40 percent carbon dioxide. Chamber temperature was maintained at 22°C; relative humidity, between 40 and 80 percent. Immediately before the animals were killed they were anesthetized with Nembutal; the lungs were removed quickly and small pieces were fixed (*5*) and then embedded in Epon araldite resin. Thin sections of these were studied by electron microscopy. Corresponding 1-μ sections were stained with toluidine blue and observed under the light microscope. Portions of lung fixed in formalin were sectioned in a freezing microtome and the sections were stained with Sudan IV.

The granular pneumocyte has an alveolar surface covered by surfactant, a phospholipid substance which forms a film between the granular pneumocyte and the oxygen of the alveolar space. The basal aspect of the granular pneumocyte is in contact with a basement membrane, fine reticular fibrils, alveolar capillaries, and portions of interstitial cells (Fig. 1). At 5486 m, where the alveolar partial pressure is decreased by 50 percent, the oxygen content of the alveolar capillary blood that supplies the granular pneumocyte must also be decreased.

The following effects of different grades of hypoxia on the granular pneumocyte have been observed. Two

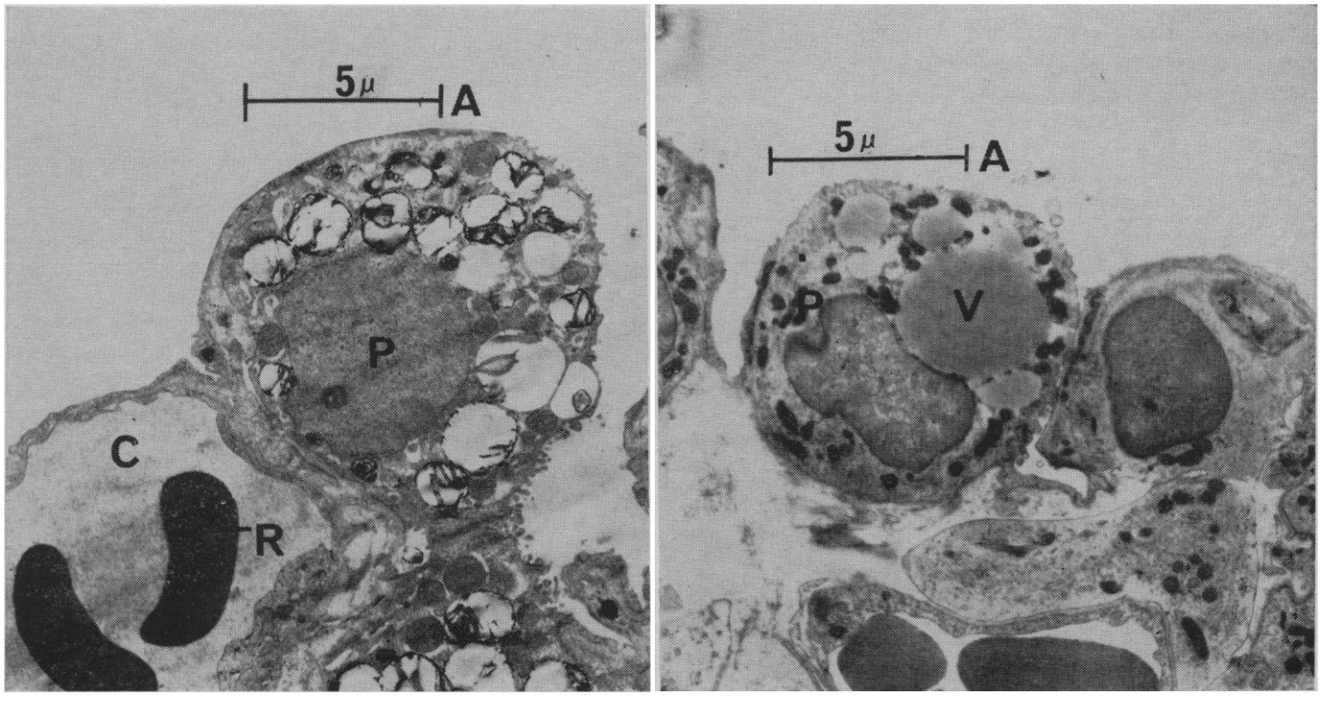

Fig. 1 (left). Lung of control guinea pig. *A*, Alveolar space; *P*, granular pneumocyte with abundant lamellar bodies; *C*, alveolar capillary; *R*, red blood cell. Fig. 2 (right). Lung of severely hypoxic guinea pig (5486 m). *P*, granular pneumocyte; *V*, lipid vacuole. Both photographs have been trimmed to show only the granular pneumocyte and adjacent structures (approximately × 5300).

animals in group 2 had small lipid vacuoles in the cytoplasm of about 30 percent of the granular pneumocytes; group 3 guinea pigs had numerous lipid vacuoles in most of the granular pneumocytes; and in animals of groups 4 and 5 the granular pneumocytes contained many large lipid vacuoles which stained with Sudan IV. However, the lamellar bodies stained only faintly. In tissue fixed in osmic acid, the lipid vacuoles were preserved but were stained only lightly with toluidine blue. The osmiophilic lamellar bodies of animals in groups 4 and 5 were smaller and fewer in number than those of the controls kept at sea level. These observations show that the fatty change of the granular pneumocyte depends on the severity of hypoxia. In hypoxia, lipid vacuoles of the granular pneumocyte are similar in morphological appearance to those of normal adipose tissue (Fig. 2). Liver and kidney sections from animals of groups 4 and 5 showed similar fat vacuoles in the hepatocytes and proximal convoluted tubule cells. These vacuoles resemble fat cysts described in cases of toxic lesions of the liver (*6*).

Monge and Singh (*7*) have described cases of high-altitude disease in which severe respiratory failure occurs and which may be related to alterations of pneumocytes. The hypoxia-induced fatty change in the granular pneumocyte indicates that this cell accumulates lipid material which may be a precursor of surfactant. Surfactant alterations have not been studied in hypoxic adult individuals. The accumulation of lipid material in the pneumocyte must be considered a vacuole or inclusion. These vacuoles may be replacing the lamellar bodies and do not resemble degenerative cellular vacuoles, cytolysosomes, cytosomes, or lysosomes. For unlike cytolysosomes and lysosomes, which contain residual membranes and myelin figures, hypoxic lipid inclusions contain a homogeneous material.

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Thermal Stability of Threonine in the Presence of a Marine Polyphenolic Material

Abstract. *The rate of decomposition of threonine in 0.01*M *aqueous solution is unaffected by an equimolar concentration of pyrocatechol, but is increased by the addition of an extracellular polyphenolic material (3 mg/ml) produced by* Fucus vesiculosus. *Glycine, a pyrolytic product of threonine, behaves similarly.*

Some analyses of fossil amino acids in hydrolyzates of carbonate exoskeletons, bone, and shale have yielded evidence of a selective diagenetic destruction of amino acids; for example, a more rapid destruction of serine, threonine, and phenylalanine than of glycine, alanine, valine, leucine, and isoleucine (*1–3*). On the basis of these data and experimental studies on the thermal stability of amino acids in dilute aqueous solution a general parallel between geological and thermal stabilities of amino acids has been proposed (*1, 3, 4*).

In other cases, however, all of the amino acids found in the proteins of living organisms have been recovered from fossil materials comparable in kind and age to those mentioned above, with little evidence of a fractional destruction (*5*). It has also been reported that the thermal stability of amino acids is greater when the acids are complexed with clay minerals than when they are present in dilute aqueous solution (*6*), and that the stability sequence obtained by the pyrolysis of natural shales differs from that based on aqueous solutions (*7*). In the latter case some of the least stable amino acids in aqueous solution (for example, serine, threonine, and ornithine) have been found to be the most stable of the amino acids naturally present in shales.

Degens *et al.* (*8*) have recently documented the occurrence of amino acids in petroleum brines. Although their analyses were largely based on methods for the determination of free amino acids, they suggested that the amino acids were not present in the free state,

Table 1. Recovery of 0.01*M* threonine (thr) and its ninhydrin-reactive pyrolytic products when heated alone, in the presence of 0.01*M* pyrocatechol (P), and in the presence of extracellular polyphenolic material (3.0 mg/ml) from *Fucus vesiculosus* (F). Concentrations in moles per 100 initial moles of threonine.

Time (min)	Contents	*p*H	Threonine (moles)	Glycine (moles)	Ammonia (moles)	N-recovery (%)
			154°C			
0	thr		100	0	0	100
0	thr + F		100	0	0	100
45	thr		100	Trace	0	100
45	thr + F		85	1	6	92
390	thr		95	3	0	98
390	thr + F		79	5	18	102
1660	thr		80	16	5	101
1660	thr + F		62	20	20	102
			174°C			
0	thr + P		100			
0	thr + F	6.60	100			
60	thr + P	5.91	92			
60	thr + F	6.39	64			
180	thr + P	5.95	79			
180	thr + F	6.62	53			
			199°C			
0	thr	6.54	100	0	0	100
0	thr + F	6.04	100	0	0	100
30	thr	6.92	54	44	4	102
30	thr + F	6.15	27	19	31	77
60	thr	7.22	28	65	2	95
60	thr + F	6.44	15	50	21	86