The fate of instilled pulmonary surfactant in normal and quartz-treated rats

Richard W. LEWIS,* John L. HARWOOD and Roy J. RICHARDS

Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, Wales, U.K.

Naturally prepared radiolabelled pulmonary surfactant can be rapidly cleared from the alveolar surface to the lung tissue after intratracheal instillation into experimental rats. This clearance is both time- and dose-dependent, a large dose (10 mg/animal) becoming associated with lung tissue more rapidly than a smaller more physiological dose (0.75 mg/animal). The data indicate that extracellular dipalmitoylphosphatidylcholine, the major component of pulmonary surfactant, is not catabolized at the alveolar surface. Alveolar free cells (mainly macrophages) appear to play a minor role in surfactant clearance. Quartz-induced phospholipidosis does not lead to an alteration in the rate of bulk surfactant clearance from the alveolar surface, although the initial distribution of the removed phospholipid complex may change in relation to the enlarged heterogenous free cell population.

INTRODUCTION

Relatively little is known about the removal and degradation of alveolar surfactant: a surprising fact in view of the great interest currently expressed in the use of surfactant-replacement therapy for the treatment of acute-respiratory-distress syndrome (hyaline-membrane disease). It has long been established that lung tissue possesses the ability to oxidize fatty acids and to degrade most lipid classes, including phospholipids (Ohta *et al.*, 1972), triacylglycerols (Geyer *et al.*, 1949) and monoacylglycerols (Wang & Meng, 1973). The turnover of pulmonary surfactant as an intact lipoprotein complex, however, presents a more complex system.

Various contrasting routes have been proposed to explain the fate/removal of surfactant or its components. These include, firstly, a bulk removal of surfactant involving carriage up the mucociliary escalator and eventual swallowing or expectoration (Notter *et al.*, 1980; Reifenrath, 1983) or clearance via the lymphatic system (Mason, 1976; Oyarzun *et al.*, 1980). Secondly, it has been proposed that lung surfactant can undergo degradation at the alveolar surface, mainly through phagocytosis by alveolar macrophages (Naimark, 1973; Stern *et al.*, 1986). Evidence in support of this includes the presence of intracellular myelinic swirls detected within the macrophage (Nichols, 1976) and the phospholipase activity found in macrophages (Elsbach & Rizack, 1963; Franson *et al.*, 1973). A recent study *in vivo* has also demonstrated the possibility that rapid (half-life < 9 min) surfactant lipid degradation, mediated by secreted phospholipase, may take place at the alveolar surface (Miles *et al.*, 1985). The speed of this postulated process would make it of great potential importance when considering the removal/breakdown of exogenously administered surfactants (as in replacement therapy or experiments on surfactant catabolism) or endogenous surfactant pools. The third possibility concerning the fate of pulmonary surfactant is the re-uptake of this material by lung tissue itself. Appreciable flux of radiolabelled dipalmitoylphosphatidylcholine (Geiger *et al.*, 1975; Hallman *et al.*, 1981) and of intact surfactant (Desai *et al.*, 1978) has

been demonstrated from the alveolar lumen into the cells of the lung tissue (cf. Jobe & Jacobs, 1984). Localization of radiolabelled phospholipids (Hallman *et al.*, 1981), and specific-radioactivity measurements in time-course experiments, have also demonstrated the bidirectional flux of material between the alveolar fraction and the type-II-cell lamellar bodies. It has also been shown that the uptake of liposomal phosphatidylcholine by cultured type II cells is both time- and phosphatidylcholine-concentration-dependent (Chander *et al.*, 1983), and that type II cells *in vitro* accumulate mixtures of radiolabelled phosphatidylcholine and unlabelled surfactant (Tesan & Bleasdale, 1985; Bleasdale & Thakur, 1986).

The role of altered surfactant degradation and/or removal in pathological conditions characterized by alveolar filling and surfactant accumulation, such as human alveolar lipoproteinosis (Larson & Godinier, 1965), or experimentally induced quartz-mediated phospholipidosis (Heppleston *et al.*, 1974) has been investigated. However, in neither of these studies was the rate of surfactant degradation directly measured.

Therefore the aims of the present study were twofold. Firstly, to investigate surfactant catabolism at the alveolar surface by monitoring the breakdown of endogenous radiolabelled extracellular dipalmitoylphosphatidylcholine (as described by Miles *et al.*, 1985) and to examine in detail (using a radiolabelled pulmonary-surfactant preparation; Lewis *et al.*, 1986) the fate and distribution with time of an instilled natural alveolar surfactant preparation. A knowledge of the mechanisms and/or routes of surfactant removal or degradation at the alveolar surface is of obvious importance in replacement therapy. With this in mind, attention was given to the effect of dose of the instilled complex on its clearance. The second aim of this work was to compare directly the rate of removal and distribution of the intact lipoprotein complex in normal animals and in those where lipoproteinosis (characterized by surfactant accumulation) had been induced by quartz instillation. The latter studies were able to make use of information concerning the optimal dosage which was established in the first part of the investigation.

EXPERIMENTAL

Materials and methods

Phospholipid standards were purchased from Sigma (London) Chemical Co., Poole, Dorset, U.K., and checked for purity by t.l.c. before use. [methyl- ^{14}C]-Choline chloride (2.15 GBq/mmol) was obtained from Amersham International, Amersham, Bucks, U.K. Fluothane was supplied by ICI, Alderley Park, Macclesfield, Cheshire, U.K. All other chemicals were of the best available grade and purchased from BDH, Poole, Dorset, U.K., or from Sigma. The DQ $_{12}$ quartz sample was kindly given by Dr. C. J. Hardy, Huntingdon Research Centre, Huntingdon, Cambs., U.K. Male specific pathogen-free (COBS-CD) rats were purchased from Charles River, Manston, Kent, U.K. All the animals were housed in wire-bottomed cages and allowed food (Pilburys Small Animals Diet; Pilburys Ltd., Birmingham, U.K.) and water *ad libitum*.

Instillation procedure and isolation of radiolabelled surfactant

Quartz samples (5 mg), sterilized by heating at 140 °C for 2 h, were suspended in 0.5 ml of sterile 0.15 M-NaCl and sonicated before instillation. Control animals received 0.5 ml of sterile 0.15 M-NaCl only. Animals were anaesthetized with Fluothane, and the dust instilled via the trachea as previously described (Richards *et al.*, 1981). To prepare radiolabelled surfactant, 0.5 ml of sterile 0.15 M-NaCl containing 5 μCi of [methyl- ^{14}C]-choline chloride was injected into anaesthetized control rats via the penal vein. Pulmonary surfactant was isolated 8 h later by the method of Abrams (1966), as modified by Harwood *et al.* (1975). Pulmonary surfactant obtained in this manner was characterized chemically and by electron microscopy as described previously (Lewis *et al.*, 1986).

Analysis of alveolar fractions from instilled animals

After treatment with radiolabelled surfactant by instillation, animals were killed by Nembutal anaesthesia, the thorax opened and the trachea clamped to prevent entry of blood into the lungs. Residual blood was cleared from the pulmonary circulation by perfusion of the pulmonary artery with 0.15 M-NaCl. The lungs were removed, the trachea cannulated and lavaged with 6 $\times$ 10 ml portions of 0.15 M-NaCl. Lung lavage fluid was centrifuged (300 g, 20 min) to sediment the free-cell population, which was resuspended and washed in 10 ml of 0.15 M-NaCl and re-centrifuged under the same conditions. The pooled supernatants were designated 'alveolar surface' fractions. All centrifugations were carried out at 4 °C.

Radioactivity counting

Lavage fluid minus free cells ('lung surface' fraction) was counted for radioactivity in PCS scintillant (Amersham-Searle; see below). The two remaining lung fractions, namely lung tissue and free cells, were homogenized (Polytron) in 0.15 M-NaCl and digested in Soluene-350 tissue solubilizer (Packard Instrument Co.) for 3 h at 50 °C. Any residual colour was bleached by the addition of 0.2–0.6 ml of 20% (w/v) H_2O_2 . After cooling, the digested samples were dissolved in PCS and counted for radioactivity. All radioactivity counting was

Fig. 1. Time course for catabolism of endogenous-surfactant dipalmitoylphosphatidylcholine

Results of experiments carried out between 8 and 30 h after precursor injection are compared with the findings of Miles *et al.* (1985) at a single 22 h time interval. Bars indicate range values of duplicate determinations (four animals per time period after injection). Results are expressed as a percentage of the radiolabelled dipalmitoylphosphatidylcholine (DPPC) measured at zero time (100%).

performed using an Intertechnique SL4000 liquid-scintillation counter. Quench correction was made by the addition of an internal standard of [^{14}C]hexadecane.

Assessment of 'surfactant' dipalmitoylphosphatidylcholine catabolism at the lung surface

Male COBS CD rats were anaesthetized (as described above) and injected with 15–20 μCi of [^{14}C]choline chloride via the penal vein; the animals were then allowed to recover and were later killed by pentobarbitol injection at various time intervals (8–30 h) after injection. The lungs were removed, after perfusing the pulmonary circulation *in situ*, and repeatedly lavaged (four times) with the same 5 ml aliquots of sterile 0.15 M-NaCl at 4 °C. The pooled lavaged fluid from four animals for each time point was maintained at 4 °C, and the free cells removed (300 g, 20 min) by centrifugation. The lavage material (pH 7.5) was incubated at 37 °C for various times (as described by Miles *et al.*, 1985), after which duplicate aliquots were removed and the dipalmitoylphosphatidylcholine fraction was separated by

Fig. 2. Distribution of [^{14}C]choline-labelled pulmonary surfactant with time after instillation

Results are means for two animals per time period; range values are indicated by the bars. Results are expressed as a percentage of total recovered radiolabel. The percentage total recovered refers to the amount of radiolabel found in all fractions examined as a proportion of the total radioactivity intratracheally instilled. The specific activity of the instilled material was 350 d.p.m./mg of surfactant; 10 mg was instilled per animal. Total recoveries were: t_0 , 75%; t_{15} , 85%; t_{30} , 62%; t_{60} , 56%; t_{180} , 41%; t_{360} , 46%; (t_0 refers to time the animal was killed; this can be up to 10 min after instillation; see the text).

the two-dimensional t.l.c. method described by Gilfillan *et al.* (1983). The bands were scraped off and radioactive counting was carried out in PCS scintillant as described above.

Chemical determinations

Lipids were extracted from tissue and from cell samples by the method of Garbus *et al.* (1963) and the phospholipid classes separated as previously described (Harwood *et al.*, 1975). The radioactivity associated with individual phospholipids was measured as described previously (Lewis *et al.*, 1986).

RESULTS

Surfactant dipalmitoylphosphatidylcholine catabolism at the alveolar surface

The contribution of surfactant lipid catabolism at the alveolar surface to the overall clearance/breakdown of the lipoprotein complex was investigated by pre-labelling surface dipalmitoylphosphatidylcholine with [^{14}C]choline chloride *in vivo* for between 8 and 30 h and monitoring the fate of this radiolabelled species in a lung lavage preparation during incubation *in vitro* at 37 °C (Miles *et al.*, 1985). Figs. 1(a)–1(d) show the results of the incubations for 0, 5, 10, 15 and 150 min after prelabelling for 8, 12, 24 and 30 h respectively. The results are expressed as percentages of the dipalmitoylphosphatidylcholine present at zero time. It is clear that, in the present experiments, no endogenous surfactant dipalmitoylphosphatidylcholine catabolism occurs at the alveolar surface for up to 150 min *in vitro* over the range of prelabelling times studied. Water-

soluble [^{14}C]choline products were not detected in any of the experiments. These data can be contrasted with the results of previous studies (Miles *et al.*, 1985; Fig. 1e), which ascribed a half-life of less than 9 min to dipalmitoylphosphatidylcholine at the alveolar surface.

Effect of dose on the removal of instilled surfactant

In order to study the effects of the instilled dose on surfactant removal with time from the alveolar surface, several experiments were carried out. In the first study, instilling a large dose (10 mg/rat) of radiolabelled pulmonary surfactant (Fig. 2) gave rise to a rapid uptake of radiolabel into the lung tissue. After 3 h, over 50% of the recovered radiolabel was found associated with lung tissue, this value rising to 65% at 6 h after instillation. There was a corresponding decrease in the percentage of recovered radiolabel detected at the alveolar surface during this time. The free cells obtained by lavage (mainly alveolar macrophages) only contained a small amount of radioactivity at all times, accumulating less than 10% of the recovered radioactivity even 6 h after instillation. Total recovery of instilled radioactivity, in the fractions examined, was found to decrease with time after instillation (75% at zero time to 46% 6 h after instillation).

In the second experiment, a much smaller, more 'physiological', dose of radiolabelled pulmonary surfactant (0.75 mg/animal) was instilled on to the alveolar surface of normal rats. This dose represented a 75% increase in normal surfactant levels, which are approx. 1.0 mg/250 g rat (Richards & Curtis, 1984). The results obtained (Fig. 3) were quite different from those found in the previous experiment. Despite the large variation noted in total percentage recovered, there was little, if any, difference in the distribution of radiolabel

Fig. 3. Distribution of [^{14}C]choline-labelled pulmonary surfactant with time after instillation

Results are expressed as means of duplicate determinations on pooled samples; bars indicate range values. Results are expressed as a percentage of total recovered radiolabel (cf. Fig. 2). The specific radioactivity of the instilled material was 22000 d.p.m./mg of surfactant; 0.75 mg was instilled per animal. Total recoveries were: $t15$, 53%; $t30$, 20%; $t60$, 40%; $t150$, 53%.

with time, into the lung tissue or the alveolar free cells, up to 3 h after instillation. If the latter time period (where total recoveries are comparable with those of the first study) is selected to compare the results from the two studies, then rats given the 0.75 mg dose of surfactant (Fig. 3) retained a higher proportion of recovered radiolabel (75%) at the alveolar surface than those exposed to the 10 mg dose (42%; Fig. 2). Conversely, rats treated with the low dose have a smaller proportion (13%) entering the lung tissue than those exposed to the higher dose (52%) at 3 h.

The implication is, therefore, that if increasing amounts of surfactant are instilled on to the lung surface, then removal is accelerated into the lung tissue. No significant radioactivity could be detected in the hilar lymph nodes of the experimental animals (to account for the percentage of the total instilled radioactivity not recovered). The results show that the pattern of uptake (and distribution in well-lavaged lung) of surfactant varies with time and dose. Examination of the data obtained after a small dose was instilled (Fig. 3) shows that less surfactant material is associated with the lung tissue at 3 h than is detected 15 min after instillation. These data would confirm that any time-dependent non-specific absorption, which could contribute to the levels of tissue-associated radioactivity detected, is of minor importance. It should also be noted that the lavage procedure used has been shown to be quantitative for the alveolar pool of natural surfactant (cf. Harwood & Richards, 1986).

In order to establish a dosage profile for the fate of instilled surfactant material, a further study was carried out in which dosage was varied and distribution monitored 3 h after instillation. These data (Fig. 4) are in agreement with the results of the first two experiments in that a direct relationship can be seen between the amount of instilled surfactant and the proportion of this material recovered in lung tissue 3 h after instillation. The higher the instilled dose then the more rapidly surfactant accumulates in this lung compartment. Conversely, the lower the instilled dose

Fig. 4. Effect of instilled [^{14}C]surfactant dose on distribution 3 h after administration

Results are expressed as a percentage of the total recovered radioactivity ($\pm$ S.D., $n = 4$) or range values (for 10 mg and 0.75 mg doses $n = 2$). Specific radioactivities of instilled material were: 0.75 mg; 22000 d.p.m./mg of surfactant; 1.0 mg, 16230 d.p.m./mg; 4.0 mg, 4300 d.p.m./mg; 8.0 mg, 1700 d.p.m./mg; 10.0 mg, 350 d.p.m./mg. Total recoveries (at 3 h) were: 0.75 mg, 53%; 1.0 mg, 30%; 4.0 mg, 24%; 8.0 mg, 24%; 10.0 mg, 41%.

then the higher the percentage remaining at the alveolar surface. This relationship is seen to hold true across a range of dose levels.

Although total recoveries were variable, decreasing from a maximum of 85% ($t15$, Fig. 2), analysis indicated that all the recovered radioactivity detected in the various fractions was associated only with phosphatidylcholine at every time interval studied (results not shown). It should also be noted that ' $t0$ ' refers to the time the animal was killed, and that up to 10 min can be allowed for dissection and removal of the lungs before lavage. Metabolism of [^{14}C]choline-radiolabelled phosphatidylcholine would lead to radiolabelling in only one other phospholipid, namely monoacyl-phosphatidylcholine. No increase in the levels of this possible breakdown product could be detected in free cells or in lung tissue.

Surfactant removal in quartz-treated animals

Quartz administered by intratracheal instillation causes a progressive accumulation of alveolar surfactant. At 5 weeks after treatment, 25% more surfactant can be isolated from quartz-treated lungs (Table 1). At 15 weeks after exposure to quartz, mean surfactant levels are five times those observed in control rats. In the total lung

Table 1. Effect of quartz treatment on lung weight, free cells and alveolar surfactant

Mean values ($\pm$ S.D.) for four animals per group are given. Surfactant data were derived from pooled samples. * denotes significant difference by Student's *t* test for unpaired samples ($P < 0.05$).

Treatment (time period)	Lung weight (g)	$10^6 \times$ no. of free cells/lung	Surfactant (mg/lung)
Control (5 weeks after instillation)	1.63 (± 0.04)	23.9 (± 4.7)	0.80
DQ ₁₂ quartz (5 weeks)	1.50 (± 0.23)	30.5 (± 11.9)	0.97
Control (15 weeks)	1.60 (± 0.15)	19.9 (± 5.2)	0.62
DQ ₁₂ quartz (15 weeks)	2.17* (± 0.36)	65.1* (± 36.3)	2.9

(and when expressed relative to wet weight), free cell numbers also show a progressive elevation with time after quartz exposure. Total lavaged lung wet weight is also significantly elevated in the quartz-treated groups 15 weeks after the single instillation. Thus a progressive lipoproteinosis has been induced, establishing a valid experimental model in which to investigate the role of pulmonary-surfactant removal in disease states characterized by surfactant accumulation.

When the distribution of 1 mg of exogenous instilled radioactive surfactant was monitored 5 weeks after quartz treatment (Fig. 5), no significant differences were detected in either the distribution or the total recovery of the instilled material, in treated, as compared with control, groups. However, 15 weeks after treatment, quartz-exposed lungs had significantly (65%) less radiolabel remaining at the alveolar surface when compared with control animals. In addition, in the whole lung the free cell population derived from quartz-exposed lungs had accumulated significantly more (fivefold) radiolabel when compared with the total free cell population from control lungs (Fig. 5). The amount of radioactivity taken up into quartz-treated lung tissue 15 weeks after exposure was elevated (but not significantly). No significant difference was detected in the total amount of radiolabel recovered from the control and quartz-treated animals.

When the rate of uptake of radioactivity into free cells and into lung tissue relative to a given number of free cells or mass of lung tissue, respectively, is calculated (results not shown), quartz treatment does not cause any significant change in either of these parameters at any time after exposure.

DISCUSSION

Any rapid breakdown of endogenous surfactant lipids at the alveolar surface mediated by secreted phospholipase activity could represent a complicating factor in the interpretation of data derived from monitoring the fate of an exogenous radiolabelled surfactant preparation. In the strain of experimental rats used in the pres-

Fig. 5. Distribution of radiolabel in control and quartz-treated animals 3 h after [14 C]surfactant instillation

Results are shown for (a) 5 and (b) 15 weeks (After instillation of 1 mg of exogenous radiolabelled surfactant) and represent (means $\pm$ S.D.) for four experimental animals per group. $\square$, control; $\blacksquare$, quartz-treated. *denotes significant difference by Student's *t* test for unpaired samples ($P < 0.05$). Total recoveries were not significantly different from control values at either time after instillation. [35 (± 11)% control; 40 (± 18)% treated at 5 weeks and 50 (± 22)% control; 39 (± 13)% treated at 15 weeks].

ent investigation, endogenous-surfactant dipalmitoylphosphatidylcholine catabolism could not be detected at the alveolar surface, in contrast with the results of previously reported studies by Miles *et al.* (1985). However, the same investigators found no evidence of endogenous dipalmitoylphosphatidylcholine catabolism at the alveolar surface in the rabbit, mouse or guinea pig (Miles *et al.*, 1985). The failure to detect any extracellular alveolar surfactant catabolism implies that removal of the (intact?) lipoprotein complex from the alveolar surface is a central prerequisite to intracellular surfactant breakdown or re-utilization, in agreement with the conclusions drawn from experiments with various radiolabelled exogenous lipids (cf. Jobe & Jacobs, 1984). Thus the use of an exogenous radiolabelled natural surfactant preparation is considered a valid experimental model with which to monitor the fate (and

particularly the uptake into lung tissue) of the complex in both normal and pathologically altered lungs.

The results indicate that the removal of exogenously administered natural pulmonary surfactant with time is dose-dependent. The larger the instilled dose the greater the amount removed from the alveolar surface of normal animals. Whether this observation reflects the ability of the lung to remove (or degrade) abnormally large amounts of surfactant material from the alveolar surface, or constitutes a degree of dose-related damage to the epithelial lining layer, resulting in rapid surfactant removal from the alveolar lumen, is unclear at present.

Examination of total recoveries after instillation indicates that bulk removal of surfactant plays a role in clearance. Radiolabelled material was not detected in whole lymph nodes. Therefore, if lymphatic clearance is important, then it must result in rapid redistribution of the radiolabelled complex from the hilar lymph nodes to other body compartments.

The lung free cell population (mainly alveolar macrophages) was responsible for the uptake of only a small proportion of recovered radiolabel in all the experiments carried out with untreated rats. This result was largely independent of time after instillation and dose of surfactant and is in agreement with previous observations *in vivo* and *in vitro* (Tetley *et al.*, 1977; Desai *et al.*, 1978). The results of the present investigation confirm that there exists an appreciable flux of radioactivity from the alveolar lumen into the lung tissue (Geiger *et al.*, 1975; Desai *et al.*, 1978; Hallman *et al.*, 1981), particularly after instillation of the larger doses of radiolabelled pulmonary surfactant. However, at the lower dose levels instilled, removal of surfactant from the rat lung surface occurred at a lower rate than that demonstrated previously for rabbits (Desai *et al.*, 1978).

After instillation of radiolabelled pulmonary surfactant, the greater part of the recovered radioactivity removed from the lung surface is associated with lung tissue (and at larger doses this phenomenon exhibits time- and dose/concentration-dependency, as does the uptake of liposomal phosphatidylcholine into alveolar type II cells *in vitro*; Chander *et al.*, 1983). Complete localization of the radioactivity within phosphatidylcholine, and the failure to detect any water-soluble [14 C]choline products in either the lung or the free-cell population, indicated that, in these fractions, no appreciable breakdown of the exogenous surfactant phosphatidylcholine had taken place up to 3 h after instillation, suggesting the possibility of recycling of intact surfactant phospholipids by lung tissue. Therefore a suitable experimental model system has been developed in which to compare the rate of removal, from the lung surface, of pulmonary surfactant in normal and in pathologically altered lungs.

The apparent quartz-mediated elevated uptake of radiolabelled pulmonary surfactant by alveolar free cells may be explained by the changing number and ratio of cell types within this population in response to dust treatment (Allison *et al.*, 1966; Cohen, 1979; Morgan *et al.*, 1980). These cells may differ in their ability to engulf and degrade pulmonary surfactant at the alveolar surface. It has also been observed (R. J. Richards, unpublished work) that rat lung lavage fluid derived from quartz-treated animals and maintained *in vitro* contained some desquamated or damaged alveolar type II cells, cells of the type implicated in normal surfactant removal/degradation (cf. Jobe & Jacobs, 1984). This

observation further emphasizes the altered nature of the free-cell population after quartz treatment. Quartz treatment only leads to a quantitative change in pulmonary surfactant, the composition of this material being unaltered (R. W. Lewis, J. L. Harwood & R. J. Richards, unpublished work). Thus the metabolic fate of surfactant in quartz-treated animals could be altered by virtue of the increased removal by the enlarged free-cell population rather than because it had a different composition. Under these conditions, there would be relatively less surfactant available for recycling via lung tissue and, more significantly, by the type II cells.

Quartz instillation eventually leads to type II cell proliferation (Richards & Curtis, 1984), giving rise to an epithelial lining layer composed mainly of type-II-like cells. Thus whereas > 90% of the alveolar surface area is normally lined by type I cells, after progressive quartz exposure this layer becomes replaced by 'altered' type II cells. It is probable that different cell types of the epithelial barrier would process surfactant and surfactant products in a different manner or at differing rates. The results presented, however, indicate little difference in the total removal rate of surfactant via lung tissue in control and in quartz-exposed rats.

In summary, bulk surfactant removal from the alveolar surface occurs at similar rates in control and in quartz-exposed animals, and surfactant distribution alters only in relation to the enlarged heterogeneous free-cell population. No evidence could be found for a significant early change in the rate of surfactant removal which would account for the developing lipoproteinosis as a response to quartz instillation. Lipoproteinosis, therefore, probably results from altered lipoprotein synthesis (Richards & Lewis, 1985), such has been suggested for asbestos-induced surfactant accumulation (Tetley *et al.*, 1977), and does not arise as a consequence (at least in its early stages) of a differential increase in the rates of both synthesis and degradation (Heppleston *et al.*, 1974).

We are grateful to the Medical Research Council for financial support.

REFERENCES

- Abrams, M. E. (1966) *J. Appl. Physiol.* **21**, 718-720
- Allison, A. C., Harington, J. S. & Birbeck, M. (1966) *J. Exp. Med.* **124**, 141-153
- Bleasdale, J. E. & Thakur, N. R. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45**, 1667
- Chander, A., Claypool, W. D., Strauss, J. F. & Fisher, A. B. (1983) *Am. J. Physiol.* **245**, 397-404
- Cohen, A. B. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 2644-2647
- Desai, R., Tetley, T. D., Curtis, C. G., Powell, G. M. & Richards, R. J. (1978) *Biochem. J.* **176**, 455-462
- Elsbach, P. & Rizack, M. A. (1963) *Am. J. Physiol.* **205**, 1154-1158
- Franson, R., Beckerdite, S., Wang, P., Waite, M. & Elsbach, P. (1973) *Biochim. Biophys. Acta* **296**, 365-373
- Garbus, J., deLuca, H. F., Loomans, M. E. & Strong, F. M. (1963) *J. Biol. Chem.* **238**, 59-63
- Geiger, K., Gallagher, M. L. & Hedley-Whyte, J. (1975) *J. Appl. Physiol.* **39**, 759-766
- Geyer, R. P., Matthews, L. W. & Stare, F. S. (1949) *J. Biol. Chem.* **180**, 1037-1045
- Gilfillan, A. M., Chu, A. J., Smart, D. A. & Rooney, S. A. (1983) *J. Lipid Res.* **24**, 1651-1656

- Hallman, M., Epstein, B. L. & Gluck, L. (1981) *J. Clin. Invest.* **68**, 742–751
- Harwood, J. L. & Richards, R. J. (1986) *Mol. Aspects Med.* **8**, 423–514
- Harwood, J. L., Desai, R., Hext, P., Tetley, T. D. & Richards, R. J. (1975) *Biochem. J.* **151**, 707–714
- Heppleston, A. G., Fletcher, K. & Wyatt, I. (1974) *Br. J. Exp. Pathol.* **55**, 384–395
- Jobe, A. H. & Jacobs, H. C. (1984) in *Pulmonary Surfactant* (Robertson, B., van Golde, L. M. G. & Batenburg, J. J. eds.), pp. 271–293, Elsevier, Amsterdam
- Larson, R. K. & Godinier, K. (1965) *Ann. Intern. Med.* **62**, 292–312
- Lewis, R. W., Harwood, J. L. & Richards, R. J. (1986) *Biochem. J.* **235**, 75–79
- Mason, R. J. (1976) in *Lung Biology in Health and Disease* (Crystal, R. ed.), vol. 2, pp. 127–169, Marcel Dekker, New York
- Miles, P. R., Castranova, V. & Bowman, L. (1985) *Biochim. Biophys. Acta* **836**, 39–44
- Morgan, A., Moores, S. R., Holmes, A., Evans, J. C., Evans, N. H. & Black, A. (1980) *Environ. Res.* **22**, 1–12
- Naimark, A. (1973) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 1967–1971
- Nichols, B. A. (1976) *J. Exp. Med.* **144**, 906–909
- Notter, R. H., Tabak, S. A. & Mavis, R. D. (1980) *J. Lipid Res.* **21**, 10–22
- Ohta, M., Hasegawa, H. & Ohno, K. (1972) *Biochim. Biophys. Acta* **280**, 552–558
- Oyarzun, M. J., Clements, J. A. & Baritussio, A. (1980) *Am. Rev. Respir. Dis.* **121**, 709–721
- Reifenrath, R. (1983) in *Pulmonary Surfactant System* (Cosmi, E. V. & Scarpelli, E. M., eds.), pp. 339–347, Elsevier, Amsterdam
- Richards, R. J. & Curtis, C. G. (1984) *Environ. Health Perspect.* **55**, 393–416
- Richards, R. J. & Lewis, R. W. (1985) *Biochem. Soc. Trans.* **13**, 1084–1087
- Richards, R. J., Tetley, T. D. & Hunt, J. (1981) *Environ. Res.* **26**, 243–257
- Stern, N., Riklis, S., Kalina, M. & Tietz, A. (1986) *Biochim. Biophys. Acta* **877**, 323–333
- Tesan, M. & Bleasdale, J. E. (1985) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 1599
- Tetley, T. D., Richards, R. J. & Harwood, J. L. (1977) *Biochem. J.* **166**, 323–329
- Wang, M. C. & Meng, H. C. (1973) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 363 (Abstr.)

Received 15 September 1986/9 December 1986; accepted 12 January 1987