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Stereologic analysis of the *in vivo* alveolar type II cell response to isoproterenol or saline administration

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Summary. Previous studies have demonstrated enhanced secretion of pulmonary surfactant from type II alveolar epithelial cells following β-adrenergic stimulation. The present study was undertaken in order to provide quantitative morphologic data supporting this effect *in vivo*. Adult male Sprague-Dawley rats were injected subcutaneously with 150 mg/kg L-isoproterenol, a wide-range β-adrenergic agonist, and killed at times 0.25 - 12 hours post-injection. Other rats were similarly injected with saline, and killed at times 0.25 - 6 hours post-injection. A third group of animals was not injected, nor handled, prior to the time of death, and served as baseline controls. Stereologic analysis of the intracellular organelles of the type II celss in the animals treated with L-isoproterenol revealed a significant decrease in lamellar body volume density, indicating increased secretion of surfactant, at 0.5 - 4 hours. The rough endoplasmic reticulum volume density increased significantly at 2 - 6 hours, indicating increased synthetic activity. In contrast, the type II cells of saline-injected animals showed no significant evidence of increased secretion, but did demonstrate a large increase in synthetic activity, resulting in many large lamellar bodies at 2 and 4 hours post-injection. The results of this study provide quantitative morphological evidence of β-adrenergic stimulation of the secretion and synthesis of pulmonary surfactant secretion by type II cells of the adult rat lung *in vivo*. In addition, they suggest an enhancement of surfactant synthesis following saline injection, which is perhaps based on endogenous catecholamine release.

Key words: Stereology - Type II cell - Pulmonary surfactant - Beta-adrenergic stimulation

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Introduction

There is evidence that the secretion, and perhaps synthesis, of pulmonary surfactant by alveolar type II epithelial cells of the mammalian lung is stimulated by sympathetic nervous activity. Previous studies, of a primarily biochemical nature, have utilized intact fetal rabbits (Corbet et al., 1979), adult rabbits (Oyarzun and Clements, 1978), isolated-perfused lungs (Massaro et al., 1982), and isolated cells (Brown and Longmore, 1981) and suggested that it is specifically β-adrenergic activation which has this stimulatory effect. The present study was undertaken in order to provide morphological evidence of this stimulation, utilizing the β-agonist L-isoproterenol administered to adult rats *in vivo*. More importantly, this study quantitates, by stereologic analysis, the reaction of the type II cell to this stimulation, providing a time-course of the intracellular morphologic response to a stimulation of function, which includes an increase in the synthetic activity of these cells. In addition this study demonstrates a significant effect of saline injection on type II cell function, indicating the complexity of surfactant control and demonstrating the necessity of uninjected controls for baseline values.

Materials and methods

Young adult (250-350 g) male Sprague-Dawley rats (Charles River Labs, Wilmington, MA) were utilized for all experiments. They were housed in pairs in standard plexiglass rat cages in the Wellesley vivarium, maintained on a 12-12 light-dark cycle and fed a standard diet (Purina Rodent Chow) ad libitum. After at least a week of standardization they were transferred to our laboratory and utilized immediately. The rats were weighed and injected subcutaneously with 150 mg/kg L-isoproterenol HCl (L-iso) in approximately 0.3 ml sterile saline, or saline alone. A third, larger, group of rats were uninjected, thus they

were not handled prior to the time they were anesthetized, as below. All animals were injected so as to make the time of death between 2:00 pm and 4:00 pm.

The experimental protocols utilized in this study are summarized in Table 1. Each group of animals, at each time point, consisted of at least 3 animals, making the entire study group approximately 50 animals. At the appropriate time post-injection (Table 1) the animals were killed with an overdose (2 ml) of pentobarbital sodium. The thorax was opened and the apical and basal lobes of the right lung were removed, immersed in 2% glutaraldehyde in 0.2 M Sorensen's phosphate buffer and chopped, by hand, into approximately 1 mm pieces.

The lung tissue was degassed in a gentle vacuum and fixed for 1 hour at 4° C. The tissue was then processed for electron microscopic study utilizing standard techniques which included post-fixation in 1% osmium tetroxide for 2 hours, dehydration in a graded ethanol series and propylene oxide, infiltration in increasing concentrations of epon (Polybed 812, Polysciences Inc.) and embedding in fresh epon. Following polymerization semi-thin (0.5 μm) sections were obtained from at least 2 blocks of each lobe of each animal. These were mounted, stained with toluidine blue, and studied by bright field light microscopy.

One block from each lobe, of each animal, was then randomly selected for electron microscopic studies. Thin (approximately 60 nm by interference color) sections were obtained, mounted on 200 mesh copper grids and stained with uranyl acetate and lead citrate. These were then examined in either a Zeiss EM-9 or a Siemens 1a electron microscope, for qualitative interpretation of ultrastructural features. Each grid was then utilized for the stereologic analysis of randomly selected type II cell organelle volume densities. In this procedure, each grid was scanned, beginning in the upper left corner, and the first 7-10 type II cells encountered were photographed at a standard magnification of 4,900x. The microscope, which was always the Zeiss in this portion of the study, was calibrated periodically in order to maintain uniformity. In order to maintain as much uniformity as possible in the cells, only those cells in which the plane of section passed through the nucleus were photographed, although this excluded very few cell profiles. This procedure resulted in photographs of approximately 45 type II cells from each time point in each group (as in Table 1). These were enlarged to a print size of 7.5 x 7.65 inches and a final magnification of approximately 15,550. The photographs were then analyzed stereologically, employing the methods of Weibel (1973), in order to determine the cytoplasmic volume densities of cell organelles involved in the production and secretion of pulmonary surfactant.

The test system for the stereologic analysis was the same as previously utilized for the study of fetal rabbit type II cells (Smith et al., 1982). In this method a 0.25 inch grid was randomly applied to the micrographs. By using the intersections of the grid lines as test points, the system consisted of approximately 900 points per micrograph. The cell organelles which were analyzed in this study included lamellar inclusion bodies, rough endoplasmic reticulum, Golgi apparatus and mitochondria. Point hits falling over the structures of interest, as well as over the entire cytoplasmic compartment of the cell, were tallied for each cell. Point hits falling over the nucleus were not tallied or included. Using the equation: Point Intercept / Point Total (Pi/Pt) = Point density (Pd) = Profile Density on a Test Area (Aa) = Volume Density (Vv) (Weibel, 1963, 1972), cytoplasmic volume densities were obtained for each of the organelles of interest.

The use of this test system resulted in a calculated maximum relative error of approximately 10% in measuring the organelle of least volume density (Golgi). While it is common practice to utilize far fewer test points for the volumetry of other structures, the test system used in the present study yielded results with very minimal relative errors, a desirable outcome.

All data were analyzed using Student's t-test.

Results

The administration of L-iso had a distinct behavioral effect on the animals, making them lethargic for approximately the first six hours of the experiment. There were no discernable behavioral changes following the saline injections. The lungs of all animals appeared normal by gross examination.

Light microscopic examination of 0.05μm toluidine blue stained sections revealed evidence of increased secretion from type II cells in the lungs of animals injected with L-iso, when compared with the uninjected control group (compare Figs. 2 and 1). At 0.5 hour post-injection there were substantial amounts of pulmonary surfactant material in some alveolar spaces (Fig. 2). This pattern continued through eight hours post-injection. An additional pertinent finding was observed at 6-8 hours post-injection. At this time the type II cells contained small, numerous darkly stained inclusions, which were interpreted as lamellar inclusion bodies. The cells thus had a very granular appearance (Fig. 3).

The lungs of rats injected with saline appeared distinctly different from either the uninjected controls, or the L-iso group, although there was little to distinguish them at short post-injection time (0.5-1 hour). By two hours occasional airspaces contained substantial surfactant material, but not to the extent observed in the L-iso group at 0.5 hour. At four hours post-injection the type II cells contained substantial numbers of large lamellar inclusion bodies (Fig. 4). The type II cells of the six hour saline-injected group appeared indistinguishable from uninjected controls.

Electron microscopic observation of the type II cells of uninjected controls revealed typical morphologic features of these cells. The lamellar inclusion bodies were evenly distributed within the cells and most had fairly evenly spaced lamellae with very few dense areas. Some lamellar inclusion bodies appeared more osmiophillic and possessed more densely packed lamellae (as in Fig. 10). The rough endoplasmic reticulum of the type II cells was fairly

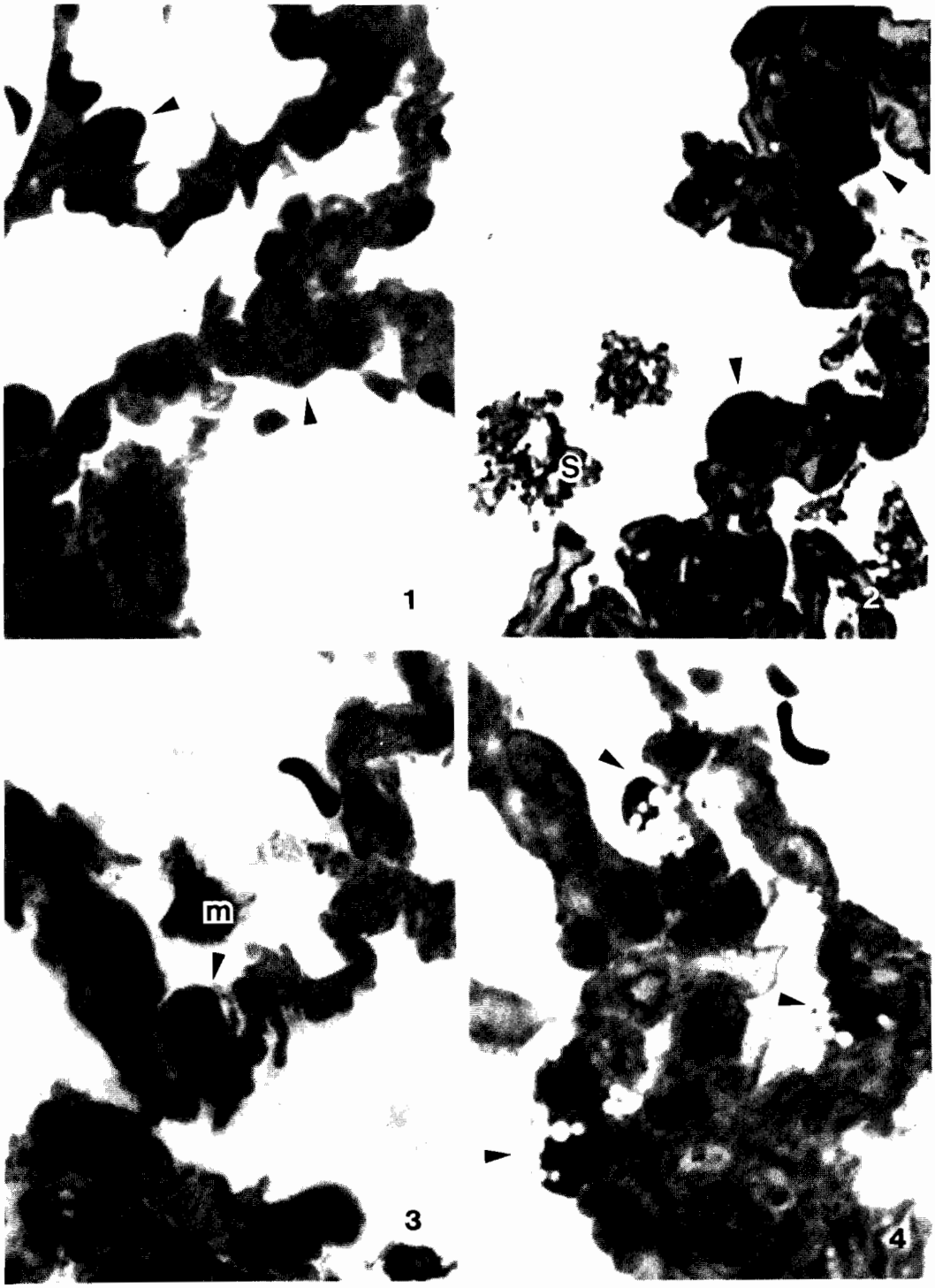


Fig. 1. Light micrograph of a toluidine blue-stained 0.5 μm thick section of rat lung from an uninjected control. Type II alveolar epithelial cells (arrows) contain lamellar inclusion bodies, a euchromatic nucleus and a prominent nucleolus. Red blood cells are evident within pulmonary capillaries. x 1,500

Fig. 2. Light micrograph of lung from the L-isoproterenol (L-iso) treated group, 0.5 hours post-injection. There are large amounts of surfactant material in the alveolar spaces at center and lower right. The type II cells (arrows) have few visible lamellar bodies. x 1, 500

Fig. 3. Light micrograph of lung from the L-iso group 6 hours post-injection. Type II cells (arrow) contain numerous small and dark lamellar inclusion bodies, giving it a somewhat granular appearance. x 1, 500

Fig. 4. Light micrograph of lung from the saline-injected group, 4 hours post-injection. The type II cells (arrows) are large and contain numerous large lamellar bodies which are somewhat pale staining. x 1, 500

abundant, consisting of thin, independent, cisternae. The Golgi was usually multiple, but not particularly abundant or obvious. The morphologic features of a typical adult rat alveolar type II cell are demonstrated in figure 5. It should be stressed that this description, and indeed all the qualitative descriptions, are of typical type II cells. There was a great deal of variation between individual type II cells in any of the groups.

The ultrastructural features of the isoproterenol-treated group were consistent with a frank stimulation of functional activity. At 0.25 hour post-injection surfactant material was found in the alveolar spaces near most type II cells. The majority of the lamellar inclusion bodies within the type II cells were found in the apical region, near the lumen. Type II cells of the 0.5 hour group sometimes contained dilted rough endoplasmic reticulum. Most had just a few small lamellar inclusion bodies, most of which were basally oriented. There were many surfactant-laden macrophages within the alveolar spaces of this group. These characteristics were also observed within the one hour post-injection group (Fig. 6). In addition to apical lamellar bodies, some cells of this group demonstrated evidence of increased lamellar body morphogenesis, namely multivesicular bodies and transitional forms.

The pattern of fewer, apical lamellar bodies continued through two hours post-injection. In addition, the rough endoplasmic reticulum of the type II cells at this point often showed evidence of increased activity. The cisternae were often distended and/or contained abundant flocculent material (Fig. 7). This was, again, not true of all cells. As demonstrated by figure 8, some type II cells of the L-iso group appeared fairly quiescent. A closer examination of figure 8, however, does reveal a great deal of flocculent material within the rough endoplasmic reticulum. The nuclei in the type II cells of this group were very euchromatic at the time points of apparent maximal synthetic activity (1-4 hours), a point well illustrated by figure 7.

At six hours post-injection, most type II cells contained basal lamellar inclusion bodies and many multivesicular bodies and transitional forms were evident (Fig. 9). At eight hours post-injection the type II cells appeared more like those of the uninjected control group, although there remained many multivesicular bodies and transitional forms. The type II cells of the 10 and 12 hour groups appeared, for the most part, like controls, athough some type II cells appeared to contain smaller, darker lamellar inclusion bodies (Fig. 10).

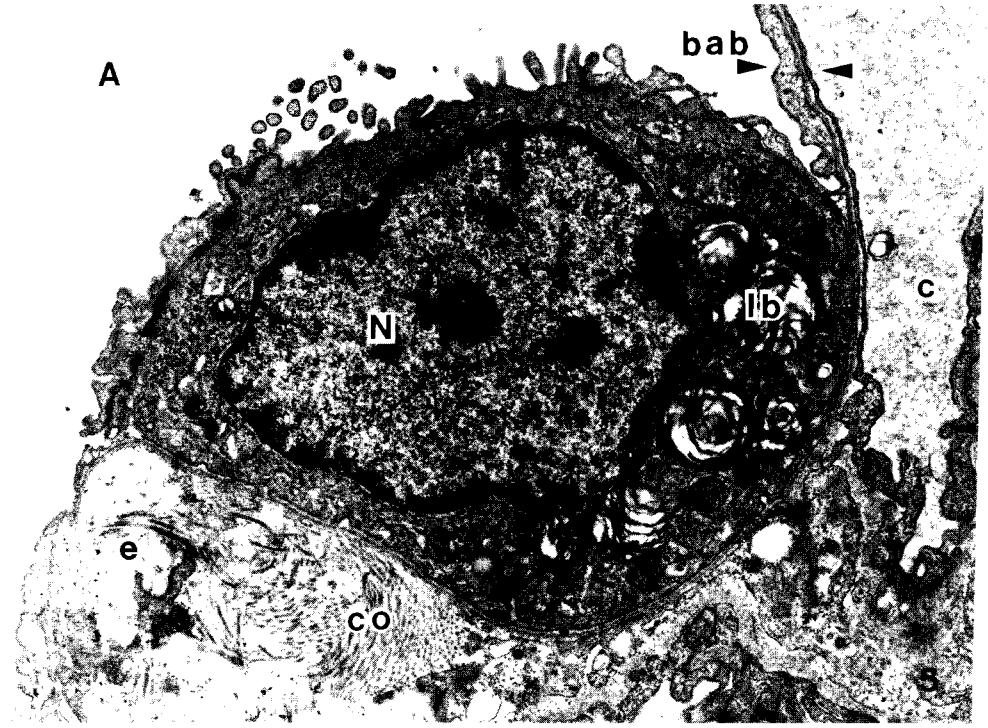
Electron microscopic observation of the type II cells of saline-injected animals revealed no obvious effects at 0.25 - 0.5 hour post-injection. At one hour some cells contained dilated rough endoplasmic reticulum, but most appeared very quiescent. The type II cells of the two hour group appeared, for the most part, large, and contained many lamellar inclusion bodies (Fig. 11). This was also true of type II cells of the four hour group. By six hours, however, most type II cells apeared indistinguishable from uninjected controls (Fig. 12).

In addition to the type II cells, interstitial fibroblasts of all groups were given particular attention. These were often found in very close apposition to type II cells, being intertwined by so-called 'foot-processes' (Vaccaro and Brody, 1981). The fibroblasts found near active type II cells (as judged by abundant, dilated RER) also appeared quite active (Figs. 7, 9, 11). There also appeared to be increased areas of extracellular matrix around some active type II cells (see Fig. 8), although this was not a consistent finding.

The results of the stereologic analysis of the cytoplasmic volume density of organelles within type II cells are listed in Table 1. The treatment with L-isoproterenol resulted in a significant decrease (when compared with uninjected controls) in the lamellar body volume density at 0.5 hour post-injection. The lamellar bodies remained significantly below uninjected control levels through four hours post-injection. At six hours the volume density increased to a level slightly, but insignificantly, above controls. It is appropriate to mention that a simple count of the number of lamellar body profiles per cell profile revealed a 40% increase at this time point. At eight hours post-injection the lamellar body volume density unexpectedly fell once again to a level significantly below control values, and remained significantly decreased at 10 hours post-injection. At 12 hours post-injection the lamellar body volume density was not significantly different from controls. The volume density analysis of the rough endoplasmic reticulum (RER) in these cells revealed a significant decrease at 0.25 and 0.5 hours after injection, followed by a steady increase to peak at four hours. However, at 6 and 8 hours post-injection the RER volume density again unexpectedly decreased sharply to levels significantly below controls, and remained depressed through 12 hours post-injection. The volume density of the Golgi apparatus was significantly decreased at one hour and at all time from 6-12 hours post-injection.

The results of the stereologic analysis of type II cells from the saline-injected rats are included in Table 1. Injection of saline caused a significant increase in lamellar body volume density at two and four hours post-injection. At six hours the lamellar bodies had returned to values not significantly different from uninjected controls. The rough endoplasmic reticulum in this group was significantly increased at one hour post-injection and decreased at six hours. The Golgi values were significantly lower at 0.25 and 0.5 hours, and again at six hours post-injection.

The results of the stereologic analyses are also presented graphically in figure 13. The initial decrease in lamellar bodies, followed by an increase, is evident in the L-isoproterenol group. In addition, the rapid, very large, increase in lamellar body volume density in the saline-injected group is also demonstrated well in this graph.



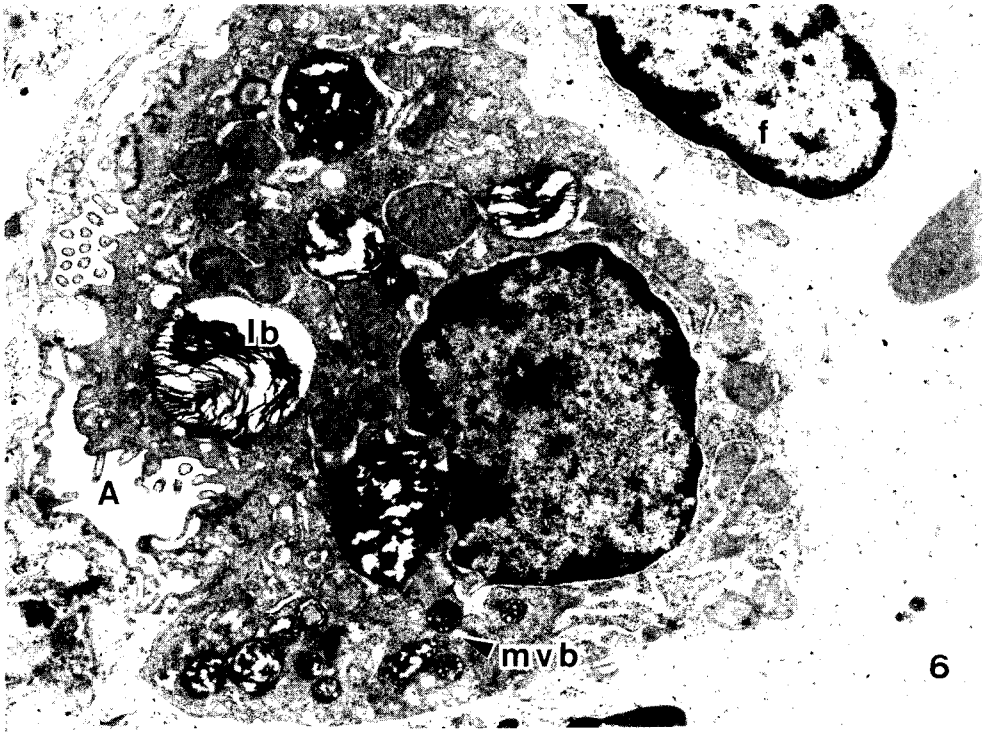
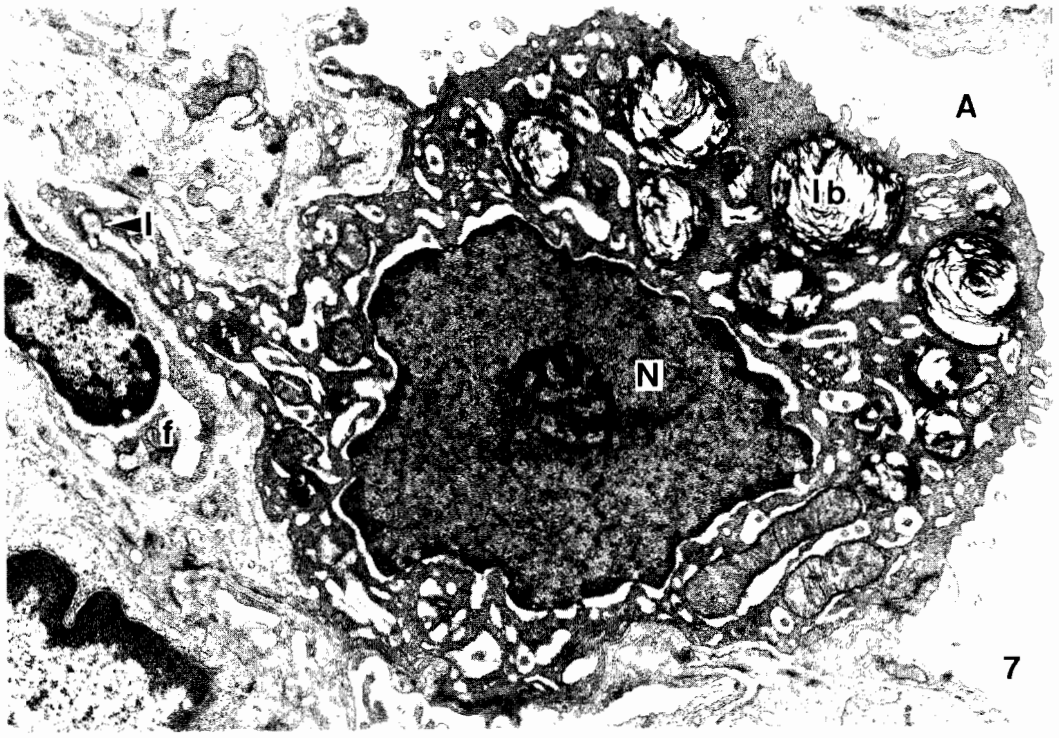


Fig. 5. Electron micrograph of a type II cell from an uninjected control rat. The lamellar inclusion bodies (1b) are evident, and contain fairly evenly spaced lamellae. The nucleus (N) is fairly euchromatic and the rough endoplasmic reticulum is scattered and not particularly evident. Note capillary space (c) and blood-air barrier (bab). Also note extensive collagenous (co) and elastic (e) fibers in the interstitium. A = alveolar space. x 11, 800

Fig. 6. Electron micrograph of a type II cell from the L-iso group, 1 hour post-injection. Many of the lamellar bodies (1b) are in an apical location. The rough endoplasmic reticulum is slightly dilated and many multivesicular bodies (mvb) are seen basally within the cell. Note the fibroblast (f) in close apposition to the type II cell. A = alveolar space. x 10, 300



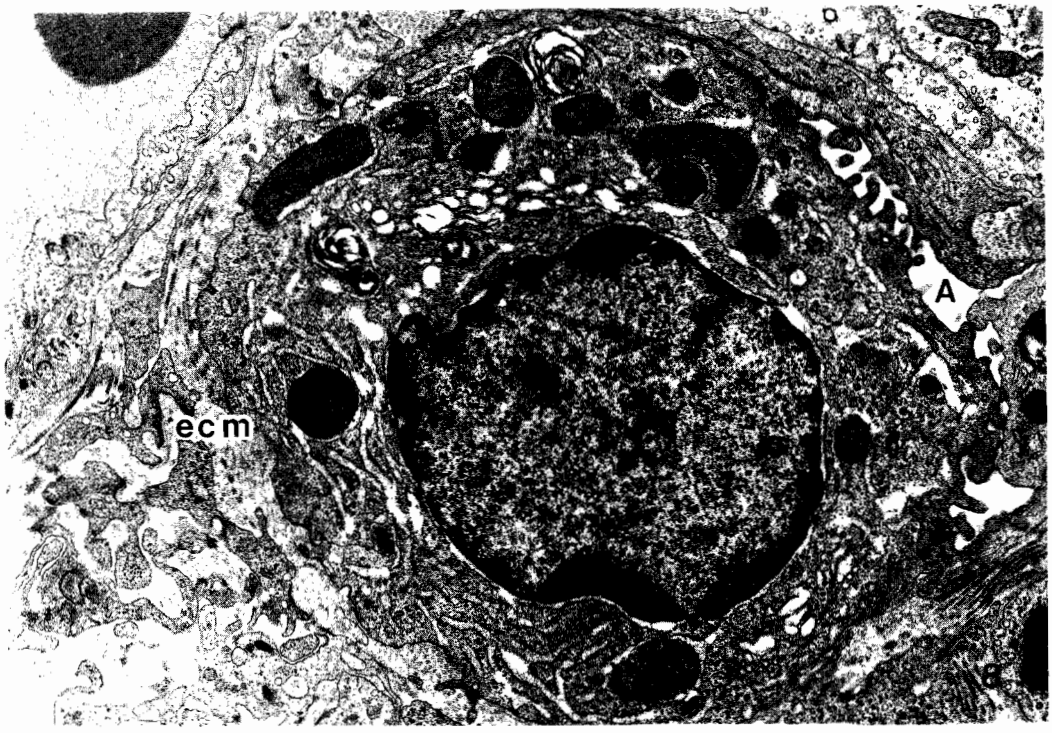


Fig. 7. Electron micrograph of a type II cell from the L-iso group, 2 hours post-injection. The nucleus (N) is extremely euchromatic. The lamellar bodies (1b) are, again primarily apical. The rough endoplasmic reticulum is markedly dilated and contains floculent material. The nearby fibroblast (f) appears somewhat activated. A= alveolar space. x 11, 800

Fig. 8. Electron micrograph of a type II cell from the L-iso group, 4 hours post-injection. This cell demonstrates the great variability between individual type II cells. While many cells at this time point appeared very active (much like that in figure 7), others seemed fairly quiescent. This cell does, however, contain quite a bit of rough endoplasmic reticulum. Note extensive extracellular matrix (ecm) around cell. A= alveolar space. x 11, 000

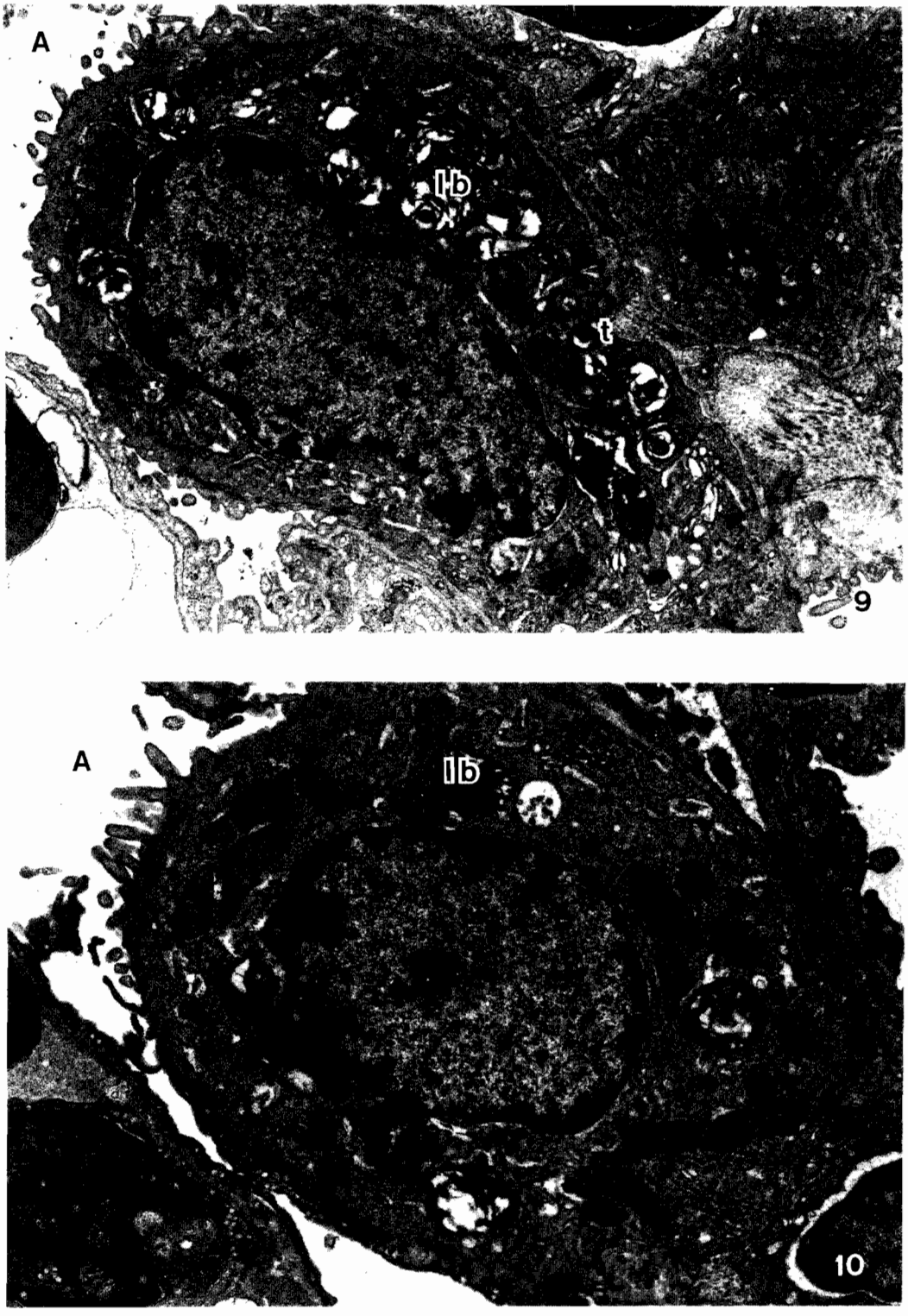


Fig. 9. Electron micrograph of a type II cell from the L-iso group, 6 hours post-injection. Most lamellar bodies (1b) are basal, with some appearing to be transitional forms (t). Note large area of fibroblast cytoplasm (f). A = alveolar space. x 12, 700

Fig. 10. Electron micrograph of a type II cell from the L-iso group, 10 hours post-injection. This cell is virtually identical to those of uninjected controls. The rough endoplasmic reticulum is inactive and the lamellar bodies (1b) are evenly distributed. A = Alveolar space. x 11, 800

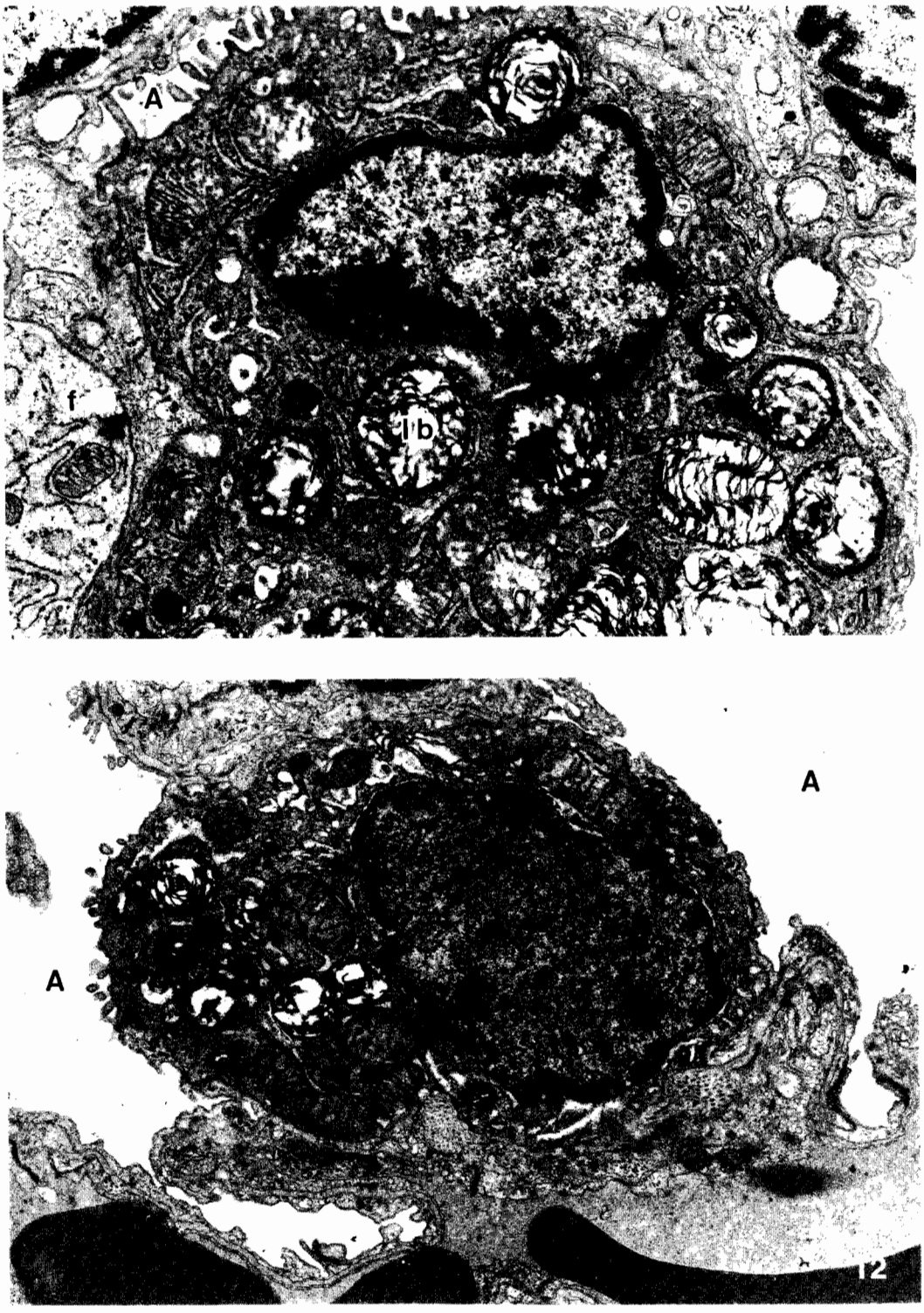


Fig. 11. Electron micrograph of a type II cell from the saline-injected group, 2 hours post-injection. This cell was very large. This photo contains approximately 60% of the entire cell. The lamellar bodies (1b) are numerous and quite large. Note the cytoplasm of an active fibroblast (f) in close proximity. A = alveolar space. x 11, 800

Fig. 12. Electron micrograph of a type II cell from the saline-injected group, 6 hours post-injection. The type II cell is indistinguishable from non-injected controls. A = alveolar space. x 11, 800

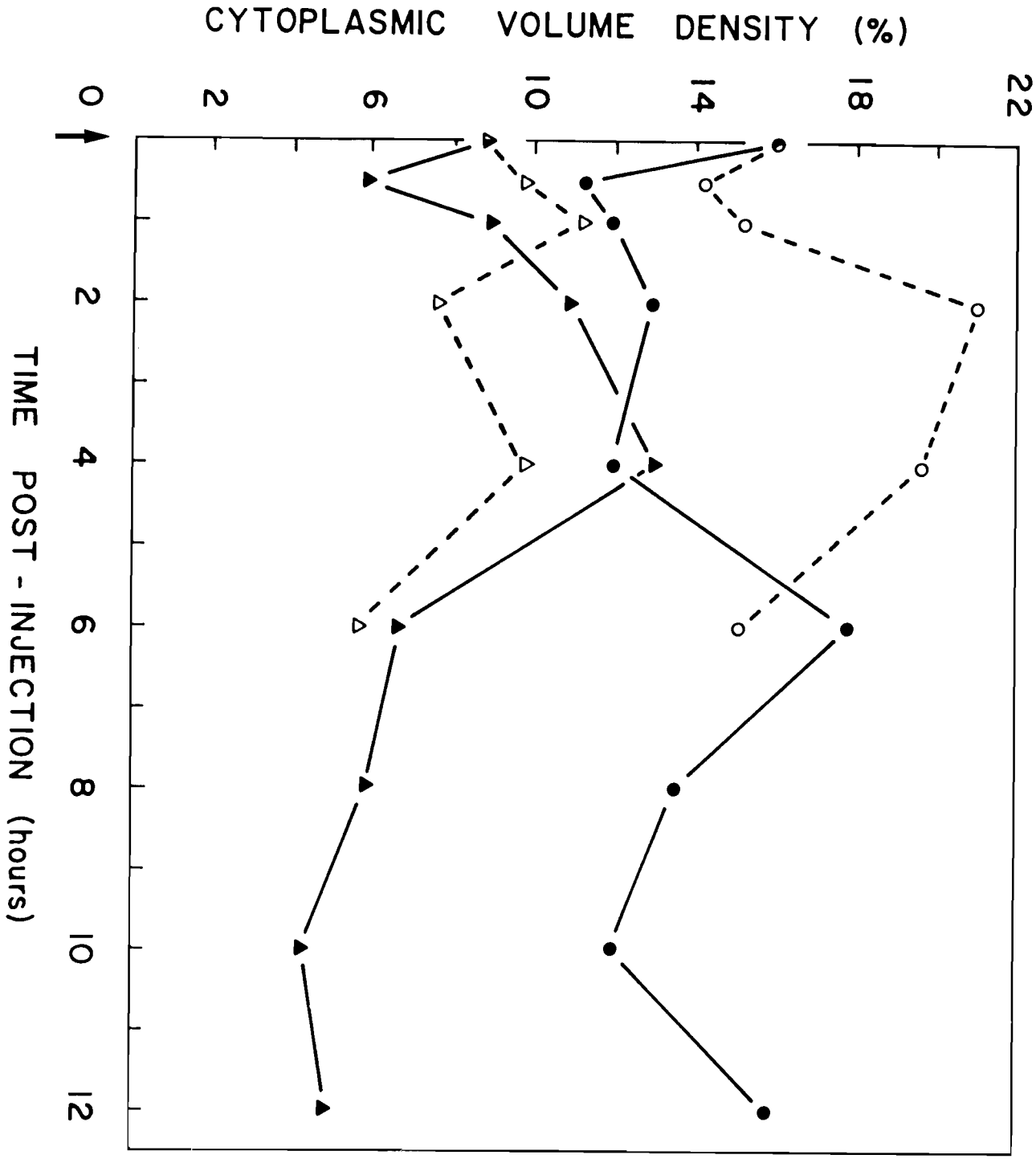


Fig. 13. Graph illustrating the results of the stereologic analysis of type II cells. Arrow indicates time zero, whose values are those of the uninjected controls.

●—● Lamellar inclusion bodies, L-isoproterenol injected.

0---0 Lamellar inclusion bodies, saline injected.

▲—▲ Rough endoplasmic reticulum, L-isoproterenol injected.

Δ---Δ Rough endoplasmic reticulum, saline injected.

Table 1. Volume density of organelles.

Group	Lamellar Bodies	Rough ER	Golgi	Mitochondria
Uninjected Controls	16.01 ± 0.74	8.83 ± 0.53	3.56 ± 0.29	12.15 ± 0.39
15 Minutes				
L-iso	15.64 ± 1.07	5.61 ± 0.36 ∅*	3.36 ± 1.87	13.21 ± 0.79
saline	13.81 ± 1.13	7.78 ± 0.47	1.78 ± 0.24 ∅	10.99 ± 0.58
30 minutes				
L-iso	11.23 ± 0.74 ∅*	5.83 ± 0.48 ∅*	3.28 ± 1.49	9.51 ± 0.52 ∅
saline	14.24 ± 0.78	9.74 ± 0.89	1.84 ± 0.21	12.02 ± 0.59
1 hour				
L-iso	11.97 ± 0.99 ∅*	8.93 ± 0.59 *	2.61 ± 0.35	12.56 ± 0.75
saline	15.24 ± 0.92	11.23 ± 0.97 ∅	3.54 ± 0.43	12.93 ± 0.61
2 hours				
L-iso	12.97 ± 1.16 ∅*	10.94 ± 0.52 ∅*	3.68 ± 0.46	12.20 ± 0.68
saline	21.04 ± 0.96 ∅	7.67 ± 0.27	3.08 ± 0.27	11.52 ± 0.46
4 hours				
L-iso	11.98 ± 0.82 ∅*	13.04 ± 0.67 ∅*	4.10 ± 0.40	12.59 ± 0.59
saline	19.65 ± 1.05 ∅	9.85 ± 0.53	3.66 ± 0.42	11.66 ± 0.59
6 hours				
L-iso	17.85 ± 0.91	6.75 ± 0.34 ∅	2.66 ± 0.23 ∅	13.59 ± 0.60 ∅
saline	15.14 ± 1.29	5.76 ± 0.24 ∅	1.76 ± 0.19 ∅	14.08 ± 0.71 ∅
8 hours				
L-iso	13.56 ± 0.86 ∅	5.89 ± 0.47	2.64 ± 0.28 ∅	13.30 ± 0.65
10 hours				
L-iso	11.98 ± 0.84 ∅	4.27 ± 0.30 ∅	2.25 ± 0.25 ∅	12.59 ± 0.59
12 hours				
L-iso	15.87 ± 0.98	4.91 ± 0.26 ∅	1.79 ± 0.17	12.87 ± 0.63

∅ Value is significantly different from uninjected control ($p < 0.5$)
* Value is significantly different from saline injected ($p < 0.05$)
N = 45 or greater for each group uninjected control N = 116

Discussion

The results of this study provide quantitative morphologic evidence for the β-adrenergic stimulation of pulmonary surfactant secretion and synthesis by adult rat alveolar type II epithelial cells *in vivo*. The time-course of this response indicates a significant stimulation through six hours post-injection. In addition, the results demonstrate a significant increase in lamellar bodies following saline injection, suggesting an enhancement of surfactant synthesis, without an accompanying increase in secretion.

In addition to the importance of demonstrating a β-adrenergic effect, the quantification of the *in vivo* morphologic response of the type II cell to stimulation is an important aspect of the present study. The results of the present study demonstrate a significant decrease in lamellar body volume density, indicating enhanced secretion from the cell. This is rapidly followed by an increase in synthesis, reflected morphologically by a significant increase in the volume density of the rough endoplasmic reticulum (RER). This quantitative increase in RER volume density was seen qualitatively as a dilation of existing cisternae. While no biochemical determination of surfactant synthesis was carried out, we feel that this dilation and filling with floculent material of the RER is an accurate and reproducible morphologic representation of increased synthetic activity within the cell. The phospholipids of pulmonary surfactant are synthesized primarily within the RER, as demonstrated by Chevalier and Collet (1972). However, it is appropriate to note that a portion of the lipid synthetic process may be catalyzed by enzymes in the cytosol (Casola and Possmayer, 1979).

There have been a number of previous studies demonstrating a β-adrenergic effect on the secretion of surfactant. The majority of these studies, however, have utilized fetal lung or type II cells in culture. Enhorning (1977) demonstrated a significant increase in surfactant secretion in near term fetal rabbits following isoxsuprine administration, but concluded that this was due to the dehydration of the lung, and not to a direct adrenergic effect on the type II cell. Corbet and co-workers (1979), again in fetal rabbits, demonstrated improved pressure-volume curves following isoxsuprine administration.

Experiments conducted in isolated type II cell preparations have provided evidence for a direct β-adrenergic effect on these cells. Dobbs and Mason (1979) demonstrated a two-fold release of disaturated phosphatidyl choline (DSPC) three hours after treatment with isoproterenol, in type II cells isolated from rats. The results of Brown and Longmore (1981) also indicated a significant

increase in the release of DSPC following isoproterenol treatment of isolated rat type II cells. Of particular interest is the fact that they could demonstrate this effect as quickly as 2.5 minutes post-treatment. In the present study, no significant difference in lamellar body volume density was observed until 30 minutes post-injection. This apparent discrepancy is most likely due to the differences between the *in vitro* and *in vivo* model systems. It is appropriate to mention that, while the *in vivo* system does have certain disadvantages, isolated type II cells in culture may be substantially different from their counterparts in the intact, *in vivo* lung (as reviewed by King, 1979). Thus, it should be appreciated that both methods are of value in the study of the surfactant system.

Previous studies utilizing the intact lung *in vivo* in the study of type II cell response to β-adrenergic stimulation are somewhat limited. Oyarzun and Clements (1978) suggested an adrenergic mechanism for the increase in surfactant phospholipid secretion they observed in response to increased ventilation in adult rabbits. Massaro et al. (1982) observed an increase in the percent of DSPC in lung lavage following isoproterenol infusion *in vivo.* They also performed a stereologic analysis which demonstrated a significant decrease in lamellar body volume density following isoproterenol treatment of an isolated-perfused lung system. While these results substantiate the findings of the present study, it is important to note that the control value obtained by this group for lamellar body volume density was only 50% of that observed in the present study. This is most likely due to the isolated-perfused methodology, since this has been demonstrated to cause a significant decrease in lamellar body volume density in the study of Defouw and Berendsen (1977).

One of the disadvantages of the *in vivo* system, that of appropriate controls, is well demonstrated by the present study. Baseline data were obtained from uninjected controls, rather than controls injected with saline. Very early in the study it became apparent that the injection of saline had a dramatic effect on the type II cells. We theorized that this effect was due to endogenous catecholamine release, based on previous reports in the literature. Buhler et al. (1978) measured plasma catecholamine levels following 30 seconds of gentle handling of rats. They observed a nearly 300% increase in noradrenaline concentrations, and a 1000% increase in plasma adrenaline. Restraint of the rat increased this to 2000% of basal (free-moving unhandled) levels.

The results of this study suggest that saline injection had a much greater impact on synthesis of pulmonary surfactant than on secretion, thus suggesting that secretion need not take place in order for an increase in synthesis to occur. This has been previously suggested by Mettler et al. (1981) who demonstrated a significant increase in phosphatidylcholine synthesis following terbutaline treatment in isolated type II cells in culture. Importantly, this effect was also seen in the presence of colchicine, which inhibited a previously observed increase in secretion. Mettler argued, and we agree, that surfactant synthesis and secretion are, at the fundamental level, independent processes.

The results of the present study do not directly address the question of the mechanism of action of the β-adrenergic agonist. The presence of β-adrenergic receptors on type II cell membrane preparations, as demonstrated by the binding studies of Sommers Smith and Giannopoulos (1983), would argue for a direct effect of the β-adrenergic agonist on the type II cells. In addition, Smith and Sidhu (1984) have recently utilized autoradiography to morphologically demonstrate specific β-adrenergic binding to type II cells *in vivo.*

In conclusion, the results of this study provide quantitative morphologic evidence of β-adrenergic stimulation of the secretion and synthesis of pulmonary surfactant by type II cells of the adult rat lung *in vivo.* In addition, they provide useful, reliable baseline values for the cytoplasmic content of adult rat type II cells, and suggest that the secretion and synthesis of surfactant within these cells are independent functions, based on the enhancement of synthesis, and not secretion, in the saline-treated animals.

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