Early Incorporation of Labelled Palmitate into Mouse Lung

There is much evidence to support the original postulate of MACKLIN[1] that the type II cell of the alveolar epithelium is the cell of origin of pulmonary surfactant and this has been reviewed by HEINEMANN[2]. There is evidence, however, that type II cells are phagocytic[3] (though not to the same extent as the alveolar macrophage), and the lamellar inclusion bodies of these cells, which are thought to be the site of secretion of the surfactant, have been shown by ETHERTON and BOTHAM[4] to contain hydrolytic enzymes of the lysosomal type, a finding difficult to reconcile with a secretory function.

There is good evidence that pulmonary surfactant is a lipoprotein, the lipid moiety of which is dipalmityl lecithin, an unusual phospholipid with considerable surface tension-lowering properties[5]. NIDEN[6] has shown by means of autoradiography that 5 min after the intraperitoneal administration of tritiated palmitate, radioactive label is found both in the type II cells and in the non-ciliated cells of the terminal bronchioles (CLARA cells[7]), and on the basis of this and electron microscopic evidence, proposes that the CLARA cells synthesize surfactant which then flows back into the alveolar lumen where it is ingested by type II cells.

This preliminary report describes the early fate of tritiated palmitate injected i.v. and traced by the technique of quantitative tissue section autoradiography.

Female mice of the Alderley Park strain weighing 25 g were used. Each animal was injected i.v. with 2.0 mC of tritiated palmitic acid (1.0 mg emulsified in 0.2 ml of a proprietary emulsifier – Dispersol OG). The animals were killed by rapid exanguination under halothane anaesthesia at $^1/_2$, $1^1/_2$, $2^1/_2$, 4, 20 and 60 min after dosing. The thorax was opened and the lungs were expanded by means of intratracheal instillation of 3% phosphate-buffered glutaraldehyde. The lungs were frozen by immersion in liquid nitrogen and 6 μm cryostat sections were cut at −20 °C, mounted on slides and dried at this temperature for 60 min. The sections were then fixed for a further 20 min in glutaraldehyde at 4 °C, thoroughly washed in distilled water, and finally dipped in Ilford K2 nuclear emulsion under darkroom conditions. The slides were placed in light-tight boxes containing a few crystals of silica gel for 8 days at 4 °C. They were then developed in Ilford IDI9B developer for 4 min, counterstained lightly with Mayer's haemalum and mounted in Zeiss phase contrast mountant. Silver grains were counted on a Zeiss photomicroscope using phase contrast, no filters, and a total magnification of 1600. Due to the difficulty of distinguishing cell membranes underneath heavy labelling, the label over terminal bronchioles was counted in groups of 15 cells (judged by numbers of nuclei), and that over the alveolar epithelium was counted in groups of 30 cells. Type II cells and macrophages were counted individually. Grain counting was performed on sections from 2 animals killed at $^1/_2$ min, 3 at $1^1/_2$ min, 4 at 4 min and 2 at 60 min.

Results. For each of the 4 time intervals that were counted, several sections of lung from each animal were examined. For every animal, counting was performed over a total of 300 CLARA cells, 300 epithelial and endothelial cells together, 100 type II cells and 50 macrophages. The background count at the edges of the slides was negligible, but in the alveolar spaces and the lumina of the terminal bronchioles it was occasionally as high as $^1/_{10}$th of the count over the cells. This was attributed to diffusion of labelled surfactant into these spaces. It was not possible to distinguish between silver grains attributable to the alveolar lining layer and those belonging to the alveolar epithelial cells, and these were included in the total count for the epithelial and endothelial cells.

Counting of silver grains over the alveolar macrophages was complicated by the presence of dust particles in some of these cells. The mean number of such particles in 100 macrophages was determined (6.752) and subtracted from the grain counts for these cells. The Table gives the mean grain counts per cell type for each of the 10 animals, and the Figure presents the combined results for all 10 animals (i.e. 3000 CLARA cells, 3000 epithelial and endothelial cells, 1000 type II cells and 500 macrophages).

Discussion. It is likely that almost all of the injected material remains as palmitate for at least the first 90 sec after injection. A proportion of this is assimilated by

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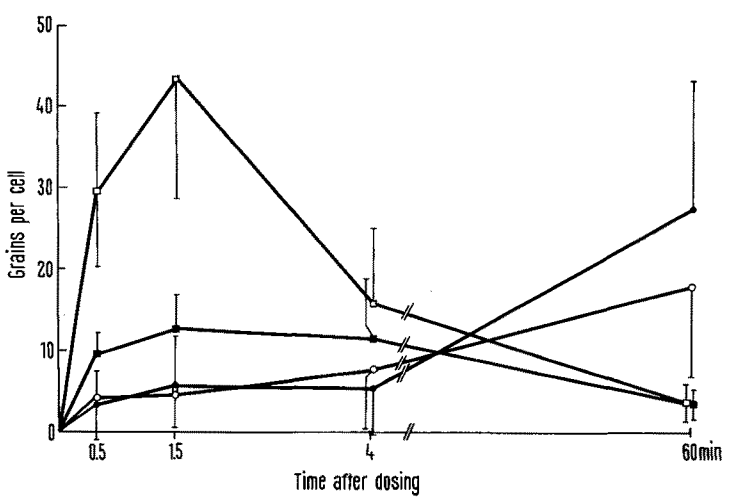

Uptake of tritiated palmitate by mouse lung cells. □, CLARA cells ●, macrophages; ■, epithelial and endothelial cells; ○, type II cells. Standard deviations are given.

Mean grain counts ± S.D. for different cell types

Time (min)	Animal No.	Clara cells	Epithelial and endothelial cells	Type II cells	Macrophages
0.5	1	33.2 ± 10.7	9.5 ± 3.1	2.5 ± 3.1	2.9 ± 5.4
	2	25.1 ± 5.3	9.6 ± 1.4	5.5 ± 3.6	4.4 ± 4.0
1.5	3	52.0 ± 12.7	10.6 ± 4.9	4.9 ± 6.3	2.5 ± 5.5
	4	39.0 ± 15.5	12.5 ± 1.9	3.9 ± 4.1	5.8 ± 7.3
	5	37.1 ± 9.9	15.7 ± 4.2	6.0 ± 2.8	7.8 ± 6.1
4	6	23.3 ± 6.9	17.8 ± 6.5	19.1 ± 9.2	7.4 ± 6.7
	7	6.0 ± 2.2	5.9 ± 2.6	3.4 ± 2.5	7.3 ± 25.7
	8	11.2 ± 5.2	3.5 ± 1.0	6.0 ± 3.8	4.0 ± 4.6
60	9	3.7 ± 2.5	4.1 ± 1.6	23.7 ± 13.7	33.2 ± 18.0
	10	2.9 ± 2.1	2.2 ± 1.0	14.3 ± 8.3	20.9 ± 9.9

the CLARA cells in the terminal bronchioles, which show significantly higher incorporation than any other type of cell ($p < 0.001$ at 30 and 90 sec). There is no difference in labelling between macrophages and type II cells at 30 and 90 sec. The inference is that during the first 2 min, the CLARA cells are actively metabolizing palmitate, and there is no uptake of palmitate from the capillaries by type II cells.

4 min after dosing the labelling of the CLARA cells is reduced, but there is still a significant difference between the degree of labelling of these and the type II cells ($p < 0.001$). Labelling of the CLARA cells and the type II cells at this stage is significantly greater than that of the macrophages ($p < 0.001$).

The alveolar epithelium (type I) and endothelium show comparable degrees of labelling over the 60 min period. The intensity was significantly greater than that seen in the macrophages and type II cells up to 4 min ($p < 0.001$) but never achieved that of the CLARA cells. This activity presumably indicates a moderately active lipid metabolism in these cells though the labelling over the type I cells may be due to newly synthesized surfactant from elsewhere. 1 h after dosing, the distribution of the radioactive source is predominantly in the macrophages and the type II cells. Macrophages show significantly more label than the type II cells ($p < 0.001$) and both kinds of cell are more heavily labelled than all the other cell types ($p < 0.001$ for both cell types).

These results may be interpreted as supporting the hypothesis that pulmonary surfactant, or more precisely, dipalmityl lecithin, is secreted by the CLARA cells and ingested at a later stage by the type II cells. The CLARA cells show at least a 5-fold increase in labelling over other cell types 90 sec after dosing, followed by a reduction in labelling during the succeeding few minutes, which suggests that the turnover of dipalmityl lecithin might be much more rapid than hitherto believed, and that secretion of this material by CLARA cells may be continuous. The type II cells nearest to the terminal bronchioles become labelled a few minutes later, suggesting that labelled material has reached them and has been ingested by them. Many of the silver grains were located over the large clear vacuoles in these cells, which are more likely therefore to be phagocytic vacuoles. Chemical analyses are underway to confirm that the tritiated palmitate remains as such during the first h of the experiment, and also to determine how much of it is incorporated into dipalmityl lecithin.

Résumé. Le taux d'incorporation de l'acide palmitique marqué à l'hydrogène 3 dans les cellules des bronchioles terminales et des alvéoles du poumon de la souris, suggère que le surfactan pulmonaire est sécrété par les cellules de CLARA dans les bronchioles terminales.

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Teratogenic Effects Induced in Tail of *Bufo arenarum* Tadpoles Following Treatment with Carcinogens

Inducing processes in the early stages of development have in particular attracted much interest. However, in later stages, very few investigations have been performed. Among chemical substances that modify the induction process, several polycyclic hydrocarbons were mentioned by BRACHET. Positive results were described by SHEN[1] in amphibian eggs and by BREEDIS[2] in *Triturus viridescens*, while NEUKOMM[3] and WOERDEMAN'S[4] investigations were not succesful.

In a previous paper we described pseudotumoral nodular formations by subcutaneous application of 20-methylcholanthrene in olive oil (MATOS and LUSTIG[6]). The experiments to be reported here deal with the teratogenic effects induced in tail of *Bufo arenarum* tadpoles following crystal implant of 3 carcinogenetic substances.

Material and method. 20-methylcholanthrene (MC), 7,12-dimethylbenz(a)anthracene (DMBA) and 3,4-benzopyrene (BP) were s.c. implanted in the middle of the tail's length with glass needles. Controls were implanted with fluorene and paraffin. No anesthesia was used. The implanted crystals remained in the site of inoculation, which appeared deficient in pigmentation. The larvae were 20–30 mm long; metamorphosis having just started, the 2 hind leg buds were visible at the time of