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This Week's Citation Classic

Kikkawa Y, Motoyama E K & Gluck L.. Study of lungs of fetal and newborn rabbits—morphologic, biochemical and surface physical development. *Amer.J. Pathol. 52:*177-209, 1968: and, **Kikkawa Y & Yoneda K.** Type-II epithelial cell of lung. 1. Method of isolation. *Lab. Invest.* 30:76-82, 1974.
[Albert Einstein College of Medicine, Yeshiva University, New York, NY: and. Boston Children's Hospital. MA]

The 1968 paper describes the relationship among changes of pulmonary Type II cells, surface-physical properties, phospholipids in the lungs, and alveolar content during fetal development and at birth. It was the foundation to prove the hypothesis that the respiratory distress syndrome of newborns occurs due to immaturity and immature surfactant development. The 1974 paper describes the method of isolating viable pulmonary Type II cells, which are used for primary cell culture from which surfactant biosynthesis and secretions have been studied. [The *SCI*® indicates that these papers have been cited more than 275 times each]

Studying the Lungs of the Young

Yutaka Kikkawa
Department of Pathology
University of California
College of Medicine
Irvine, CA 92717

After my pathology residency training, I became a junior instructor at the Albert Einstein College of Medicine. One day, I received a call from Etsuro K. Motoyama from Boston Children's Hospital. Motoyama, a fellow intern at Tokyo US Army Hospital, asked for collaboration on the work conducted under the guidance of Charles Cook, then chief of staff at Boston Children's. At the time we undertook the project, "respiratory distress syndrome of the newborn" was termed hyaline membrane disease, since it was thought that eosinophilic substance (hyaline) lining the alveolar ducts was responsible for the collapse of newborn lungs. Our collaborative study resulted in several publications[1] which set the foundation that hyaline membrane disease was caused by immaturity of newborns.

After Cook moved to Yale University, the collaboration continued with Motoyama and L. Gluck. The *Citation Classic* paper published in 1968 correlated the developmental changes of pulmonary Type II cells, lung disaturated phosphatidylcholine (principal component of lung surfactant), and surface-physical characteristics of the lungs. In my experience, this was one of the most rewarding collaborative studies I have ever done: three investigators of different expertise with one specific aim. This paper laid the foundation for the development of the lecithin/ sphingomyelin (US) ratio of amniotic fluid, now routinely used for determination of fetal maturity.

This study, however, only provided the circumstantial evidence that Type II cells may be the source of pulmonary surfactant lipids. Thus, in 1972, I undertook isolating Type II cells in a viable form from more than 40 different cell types. Our laboratory was not equipped for cell biological work then. We therefore salvaged a discarded refrigerated centrifuge, along with other equipment, and we published the data using 1,700g for isolation of Type II cells. There was no special magic for this number. Our centrifuge could only go as high as 1,700g! K. Yoneda and I, along with Akira Suzuka (EM technician) all worked about 16 hours a day for three months. By then, we had pure Type II cells. We probably carried out more than 300 experiments during this period.

After obtaining pure Type II cell pellets, we were unable to send the manuscript for three more months because we could not obtain a low power electron micrograph of isolated Type II cells. Suzuka told us that some hard material in the cell pellets ruined his knives and the electron micrographs were full of scratch marks. In those three months, we found that the Joklik solution packaged by the company was the culprit.

The isolated Type II cells have provided opportunities for direct metabolic study of two major lipid components of pulmonary surfactant.[2,3] The isolated Type II cells have been cited in numerous papers, having been used in many different fields in pulmonary biology ever since.

It is interesting to note that this paper was rejected by *Science* because "Type II cell yield was too small." I happen to know whose comment this was. This reviewer's team was funded by NIH for isolation of Type II cells for many years. My work was carried out under an NIH grant entitled "Ultrastructural Study of the Lungs."

1.Reynolds E O R, Jacobsen H N, Motoyama E K, Kikkawa Y. Craig J M, Orzales M M *&* Cook C D. The effect of immaturity and prenatal asphyxia on the lungs and pulmonary function of new born lambs. the experimental production of respiratory, distress *Pediatrics* 35:382-92. l965s.(Cited 105 times.)

2.**Smith F & Kikkana Y.** Type II epithelial cells of the lung. Ill Lecithin synthesis *Lab.Invest.*38:45-51 ,1978 .

3. ----------. Type II epithelial cells of the lung.V. Synthesis of phosphatidyl glycerol.*Lab.Invest*, 40:172-7. 1979

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