Composition of Marine Organisms (Sears Foundation for Marine Research, Yale University, New Haven, Conn., translated by J. Efron and J. K. Setlow, 1953).
10. A. E. Parr, *Bull. Bingham Oceanogr. Collect. Yale Univ.* **7**, 1 (1939).
11. E. J. Carpenter, unpublished results.
12. J. J. Goering, R. C. Dugdale, D. W. Menzel, *Limnol. Oceanogr.* **11**, 614 (1966).
13. R. R. L. Guillard and W. F. Blankley, personal communication.
14. J. K. Dooley, *Contrib. Mar. Sci.* **16**, 1 (1972).
15. K. Jones and W. D. P. Stewart, *J. Mar. Biol. Ass. U.K.*, **49**, 475 (1969); *ibid.*, p. 701.
16. I thank S. Smith, J. P. Clarner, and N. Corwin for assistance; and R. R. L. Guillard, S. W. Watson, and R. S. Scheltema for comments on the manuscript. Gratitude is also expressed to W. J. Woelkerling for confirming the identification of *D. fucicola*. Contribution No. 2922 from the Woods Hole Oceanographic Institution.

25 August 1972; revised 16 October 1972

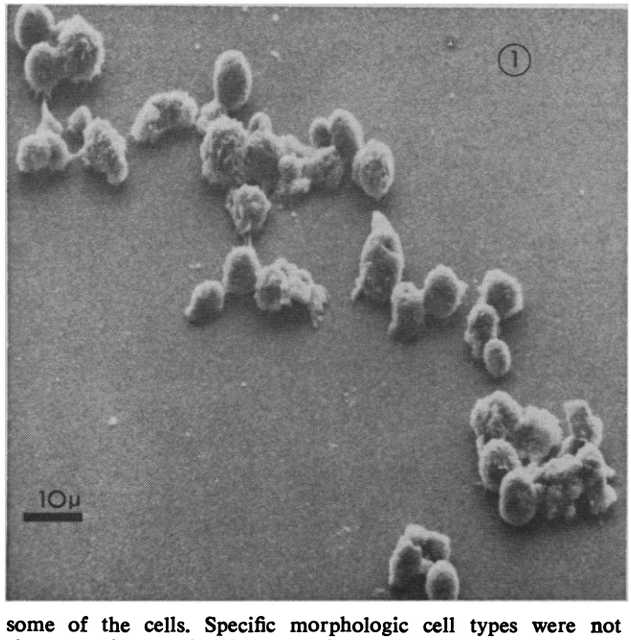
Dispersal of Rabbit Lung into Individual Viable Cells: A New Model for the Study of Lung Metabolism

Abstract. *This new enzymatic method disperses rabbit lung into morphologically intact, viable individual cells. The scattered cells constitute more than 50 percent of the original tissue. At least 90 percent of the cells exclude trypan blue from the nucleus. The dispersed lung cells consumed 6.2 microliters of oxygen per hour per milligram, dry weight. They incorporated [1-^{14}C]palmitate into lecithin.*

Evaluation of the individual metabolic characteristics of each of the many morphologically distinct types of cells that occur in mammalian lungs requires a method to disperse them into a mixed population of individual viable cells (IVC) prior to their isolation into homogeneous groups. Dispersal of other tissues into IVC has been accomplished with mechanical, enzymatic, chemical, antibody-mediated, and combined methods (*1*), but thus far no satisfactory method for the dispersal of mammalian lung has been available. This report describes a method for the enzymatic dispersal of rabbit lung into IVC that are morphologically intact, exclude a vital dye, consume oxygen, and incorporate palmitate into lecithin.

New Zealand white male rabbits (average weight, 1.7 kg) were anesthetized intravenously with sodium pentobarbital. After tracheostomy, the lungs were artificially ventilated with air, the chest was opened, and a catheter inserted through the right ventricle into the pulmonary artery was secured with ligatures. The left atrium was excised to reduce outflow obstruction to the perfusate and minimize formation of pulmonary edema. The lungs were perfused at 38°C in situ with 100 to 200 ml of Krebs (*2*) serum substitute solution III (KSSS), which is low in bicarbonate and high in phosphate, plus 2 percent fetal calf serum at *p*H 7.4. Perfusions done at flows of 60 to 70 ml/min and at a pressure of less than 20 cm-H_2O removed most of the blood cellular elements. The lungs were excised, weighed, and filled through the trachea with 80 to 100 ml of iced KSSS without calcium or magnesium but with 2 percent fetal calf serum. The solution contained, per milliliter, 1 mg of crude collagenase, 2 mg of Pronase, 0.5 mg of chymopapain, 10 units of elastase, 0.03 mg of deoxyribonuclease to reduce agglutination of cells, and 0.005 ml of crude catalase (*3*). The lungs were cut into approximately 1-cm cubes and then into slices 1 mm thick. These were incubated in the above enzyme mixture at 38°C for 60 minutes with gentle agitation at 90 cycles per minute in a Dubnoff shaker. A *p*H stat delivering 0.154*M* NaOH maintained the *p*H at 7.4. An oxygen stat delivered 0.088*M* H_2O_2 to maintain the partial pressure of oxygen (P_{O_2}) at 130 ± 10 torr (*4*).

To reduce mechanical and chemical damage to the loose cells from continued shaking in the enzyme mixture, the IVC were filtered through polyester mesh at intervals of 10, 20, 30, 45, and 60 minutes while the undispersed tissue residuum was returned to the enzyme mixture in the dispersal flask.



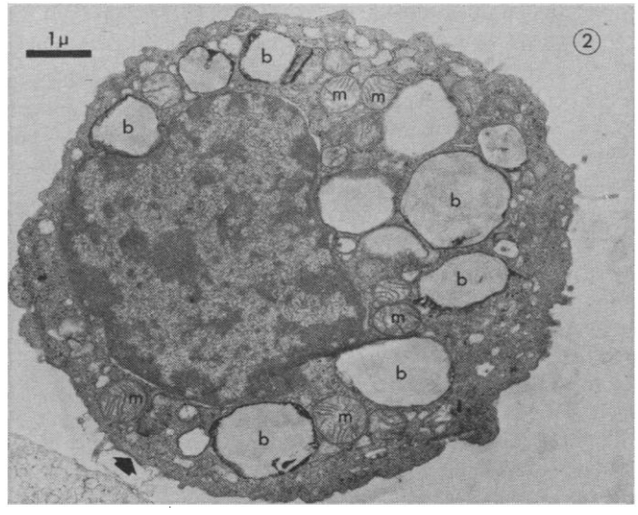


Fig. 1 (left). Scanning electron micrograph of air-dried preparation of individual dispersed lung cells coated with 30 percent fetal calf serum, which shows that the individual cells tend to be spherical or ovoid with irregular processes extending from some of the cells. Specific morphologic cell types were not identified by this method (× 800). Fig. 2 (right). Transmission electron micrograph of a granular pneumonocyte or type II alveolar cell which shows good preservation of the cytoplasmic organelles including mitochondria (*m*). The "lamellar bodies" (*b*) have lost the typical lamellate character which usually distinguishes this cell so that the electron-dense material is now located at the periphery of the cytosome thought to contain surfactant (*5*). This change occurs after perfusion to remove blood cellular elements. A small portion of the membrane of another cell remains attached (arrow) (× 9260).

Viability was estimated by determining the percentage of the IVC that excluded 0.07 percent trypan blue dye from their nuclei. The number of cells with stained nuclei progressively decreased from 12 percent in those cells initially dispersed to less than 4 percent in cells dispersed at the end of 60 minutes of incubation with the enzymes. Individual cells with active cilia were seen in the counting chambers; these were the only cells in the dispersed population which could be positively identified by light microscopy. Scanning electron microscopy showed the surface character of the dispersed cells (Fig. 1).

Granular pneumonocytes or type II alveolar cells (Fig. 2) were frequently present among the cells identified by transmission electron microscopy. The type II alveolar cell is thought to be the source of the alveolar surface active material or surfactant (*5*). We also identified the following types of IVC: ciliated, goblet, Clara, argentaffin, mucous, type I alveolar, capillary endothelial, fibroblast, smooth muscle, macrophage, and mast cells.

The yield of IVC from rabbit lung by this method is greater than 50 percent by volume of the original lung weight. Yields of this magnitude equal or exceed those obtained by most techniques for the dispersal of other tissues. This technique has also been successfully applied to the dispersal of cat, rat, and dog lungs.

The total yield of IVC from a single rabbit lung was combined in 70 ml of KSSS, 30 ml of fetal calf serum, 3 mg of deoxyribonuclease, and 0.5 ml of crude catalase. Oxygen consumption (Q_{O_2}) was determined on samples of mixed IVC in a thermostatically controlled (38°C) glass incubation chamber, and the P_{O_2} was kept at 130 ± 10 torr by an oxygen stat delivering 0.022*M* H_2O_2 (*4*). The *p*H was maintained at 7.4 ± 0.02 with a *p*H stat delivering 0.02*M* NaOH. Cells were gently agitated with a magnetic stirrer. The Q_{O_2} for the IVC averaged 6.2 μl (standard deviation 1.3) of oxygen per hour per milligram, dry weight, in ten animals. By comparison the Q_{O_2} for rabbit lung slices was reported to be 6.7 by Barron and colleagues (*6*) and 8.0 (S.D. 1.0) by Krebs (*2*). We found that IVC from rabbit lung were capable of incorporating 3 percent of added [1-^{14}C]palmitate into saturated lecithin when maintained for 90 minutes in this incubation system—a process that would correspond to formation of 5 to 10 percent of the pool of saturated lecithin if one considered it to be de novo synthesis or a deacylation-reacylation reaction utilizing unsaturated lecithin. Incorporation of labeled precursor into this major component of pulmonary surfactant (*7*) shows that the IVC can carry out an energy-requiring series of reactions at a significant rate.

KENNETH G. GOULD, JR.
Department of the Air Force, School of Aerospace Medicine, Brooks Air Force Base, Texas 78235, and *Department of Physiology, University of California, San Francisco 94122*
JOHN A. CLEMENTS
ALBERT L. JONES
JAMES M. FELTS
Cardiovascular Research Institute, Departments of Pediatrics, Medicine, Anatomy, and *Physiology, University of California, San Francisco 94122*, and *Cell Biology Section, Veterans Administration Hospital, San Francisco 94121*

References and Notes

1. A. Moscona, in *Cells and Tissues in Culture*, E. Willmer, Ed. (Academic Press, London, 1965), vol. 1, pp. 49–60.
2. H. Krebs, *Biochim. Biophys. Acta* **4**, 249 (1950).
3. Collagenase (code CLS, lot 9GB, Worthington Biochemical Co., Freehold, N.J.); Pronase (lot 000875, CalBiochem, Los Angeles, Calif.); chymopapain (code CYPAP, lot 7AC, Worthington Biochemical Co.); elastase (code E-1250, Sigma, St. Louis, Mo., and code 36-171, lot 23, Miles Laboratories, Kankakee, Ill.); DNAase I (electrophoretically purified bovine pancreas, lot V-1990, Schwarz BioResearch, Orangeburg, N.Y.); crude catalase (beef liver, lot W-1105, Schwarz BioResearch).
4. Unpublished method of M. Berry and J. Felts.
5. S. Buckingham and M. Avery, *Nature* **193**, 688 (1962); M. Klaus, O. K. Reiss, W. H. Tooley, C. Piel, J. A. Clements, *Science* **137**, 750 (1962).
6. E. Barron, Z. Miller, G. Bartlett, *J. Biol. Chem.* **171**, 791 (1947).
7. J. A. Clements, J. Nellenbogen, H. J. Trahan, *Science* **169**, 603 (1970); D. Tierney, J. A. Clements, H. J. Trahan, *Amer. J. Physiol.* **213**, 671 (1967).
8. Supported in part by grant HL-06285 from the National Heart and Lung Institute and by a grant from the Tuberculosis and Respiratory Disease Association of California. Excellent technical assistance was provided by Miss Sarah Jones. The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences–National Research Council.

17 August 1972; revised 25 September 1972

Reversed Development and Cellular Aging in an Insect

Abstract. *Larvae of the beetle* Trogoderma glabrum *undergo partially reversed development when deprived of food and water. Retrogressive larval ecdyses occur, and the larvae become diminished in size and weight. Given food, the larvae regrow, ecdyze, and regain their previous degree of maturity. Repeated cycles of retrogression and regrowth are possible. Although larval in form and organ differentiation, repeatedly retrogressed insects display a physiological deterioration suggestive of aging. A deterioration of the ability to regrow is accompanied by increasing fat body polyploidy.*

Experimentally reversed development has never been accomplished with a metazoan animal, in the sense of the animal's becoming progressively "younger" from the standpoint of physiological state or tissue differentiation. Were the genetically controlled developmental program reversed, there might also be the possibility of reversing genetically controlled aging processes. However, to the degree that aging effects are probability-related accidental damage inflicted on cell systems, they could not be forestalled by a reversal of the developmental program (*1*).

Insects are good experimental animals for research on these relationships, because their postembryonic development is divided quite sharply into larval and adult developmental sequences, with a corresponding division of the total genetic complement into larval and adult genomes (*2*). In a small number of holometabolous species, adult development can be prevented under conditions that prevent the "turning on" of the adult genome (*3*). Genetic control of aging processes should be a function of the adult genome, and if so, any aging manifested in long-lived larvae would be attributable only to time-related tissue damage. We have investigated some aspects of the problem, and have found that larval development may be reversed, at least to a limited degree. The superannuated larvae produced eventually displayed physiological dysfunctions suggestive of senescence.

Larvae of a dermestid beetle, *Trogoderma glabrum* (Herbst) were used, being reared in stock culture as previously described (*3*). Nearly mature "last" instar (sixth) larvae were removed from stock culture and isolated in small test tubes (10 by 75 mm) without food, granular substrate, or water. Under these conditions, the larvae underwent an apparent reversal of larval development. Larvae so treated did not