^{14}C-1-Palmitate Incorporation by Rat Lung: Effect of Nitrogen Dioxide[1] (34969)

THOMAS THOMAS, JR., AND RODNEY A. RHOADES
(Introduced by H. S. Weiss)
Center for Air Environment Studies and Laboratory for Human Performance Research, The Pennsylvania State University, University Park, Pennsylvania 16802

The alveolar lining of the mammalian lung contains a surface-active layer which lowers and stabilizes surface forces during the respiratory cycle (5, 20, 23). The essential component of the surface-active material which, in turn, exerts its characteristic effect on lung mechanics is dipalmitoyl lecithin (4, 17, 23) and, to a lesser extent, other phospholipids (17, 23). The high rate of lipid synthesis observed in lung tissue is believed to be involved in the continuous replacement of the phospholipids in the surface-active layer (1, 2, 12, 26); and therefore, alveolar stability ultimately depends, at least in part, on this process.

Alterations of the surface-active material have been implicated as a causative factor in the development of pulmonary emphysema, atelectasis and edema (5, 21, 24). These pathological conditions have been reported following exposure to nitrogen dioxide (NO_2), a respiratory irritant. Because pulmonary lipid synthesis is involved in the maintenance of the surface-active material, it was felt that a study of lecithin metabolism during NO_2 exposure might help clarify the NO_2 effect. Labelled palmitate incorporation into lung lecithin was chosen as the method of study because palmitic acid is the most abundant fatty acid constitutent in lung lecithin (4, 11, 17, 20).

Methods and Materials. Male Long-Evans Hooded rats averaging 295 g ($\pm$ 5.30 SD) were continuously exposed for 2 weeks to an average of 5.1 ($\pm$ 0.29 SD) parts per million (ppm) of NO_2 (Matheson Co.) in a stainless steel exposure chamber similar in design to that described by Hinners *et al.* (15). Nitrogen dioxide was monitored by a NO_2 meter (Mast Co.). Temperature and relative humidity were controlled between 72–74°F and 40–60%, respectively. Water and food were supplied *ad libitum*. Control rats (av wt = 301 $\pm$ 6.10 SD) in an identical control chamber received filtered air. Following the 2-week exposure, the air and NO_2 exposed animals (N = 12/group) were injected intravenously via the femoral vein with 5 μCi of ^{14}C-1-palmitate (New England Nuclear) bound to bovine serum albumin. Three animals from each group were sacrificed at 1, 2, 15, 24 hr after injection by intraperitoneal pentobarbital injection and exsanguination via a carotid artery.

Lipids were extracted from lung tissue by the methods of Folch *et al.* (8). Phospholipids were separated by thin-layer chromatography on extra pure silica gel H.R. (Brinkman Instruments, Inc.). The solvent system was $CHCl_3$:MeOH:H_2O:29% NH_4OH, 130:70:6.5:0.6 [v/v] (19). Five 0.25-mg aliquots were plated from each extracted sample. After separation, two lecithin spots from each sample were analyzed for radioactive content using a Unilux II liquid scintillation counter (Nuclear Chicago Co.). Samples were aspirated directly into vials containing 15 ml of scintillation fluid (4 g of PPO and 0.1 g of POPOP/liter of toluene). The remaining three lecithin spots from each sample were analyzed for phosphorus content using the method of Morrison (18). Specific activities of lung lecithin were then expressed as dpm/μg of P.

Biological half-life ($T_{1/2}$) of lecithin was determined by plotting the specific activity

[1] Supported by U.S. Public Health Service Grant No. AP00022, and NIH Grant No. ES-00335.

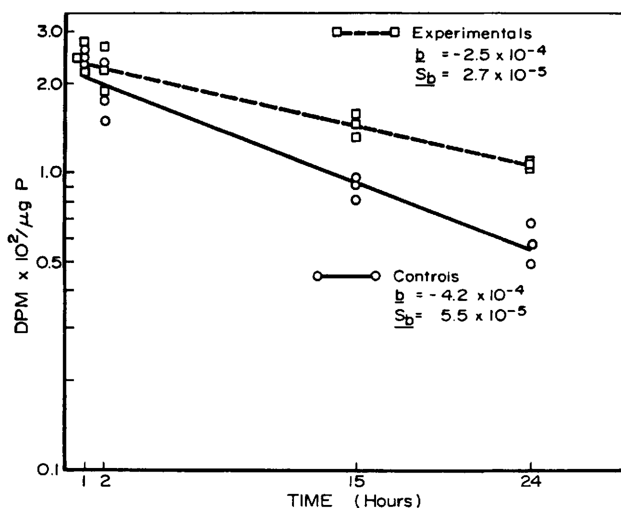

FIG. 1. Semilog plot of ^{14}C-palmitate incorporation into lung lecithin following exposure to nitrogen dioxide: b = regression coefficient; Sb = standard deviation of regression coefficient.

against time on semilogarithmic paper. Linear regression analysis was used to determine the best line of fit for each decay curve.

Results. ^{14}C-palmitate incorporation into lung lecithin was studied in rats continuously exposed to 5 ppm of NO_2 for 2 weeks. No significant difference ($p > 0.05$) in body weight existed in the animals exposed to NO_2. The incorporation of ^{14}C-palmitate into lung tissue lecithin at various time intervals after intravenous injection of the albumin complex is shown in Fig. 1. Maximum incorporation into lung lecithin for both air and NO_2-exposed animals occurred at 1 hr post-injection. Biological half-life ($T_{1/2}$) for lung lecithin was determined from decay curves. Lecithin was found to decay exponentially as a function of time in both air and NO_2-exposed animals. Biological $T_{1/2}$ for the air group was approximately 12 hr while that for the NO_2-exposed was 21 hr.

Discussion. Following maximum incorporation of ^{14}C-1-palmitate into lung lecithin, $T_{1/2}$ was markedly increased by exposure to NO_2 of lung *in vivo*. Zilversmit and co-workers (27) have shown that after labeling by a single injection of a precursor, a decay curve reflects turnover, *i.e.*, renewal of substance if reutilization of the label does not occur after degradation. Palmitate reutilization in lung lecithin apparently does not occur (26). Thus, the increase in $T_{1/2}$ of lecithin in NO_2 exposed lungs indicates a marked decrease in lecithin turnover. Although we made no measurements on lecithin synthesis, the observation that there is a marked decrease in lecithin renewal suggests that plumonary phospholipid synthesis is altered after NO_2 exposure.

In considering how NO_2 might affect phospholipid metabolism one possible explanation could be an alteration in glycolysis. Metabolism of glucose appears to be essential in phospholipid metabolism in that it provides, among other precursors, L-α-glycerophosphate for fatty acid synthesis (7, 13). A reduction of necessary precursors could account for a decreased synthesis. A second possibility could be a marked reduction in ATP levels. This could reduce esterification of labeled palmitate thus accounting for the observed turnover (7, 22). Further studies of glycolysis and ATP levels are required to clarify these possibilities.

Many investigators (5, 12, 23, 26) accept the view that cyclic lung compression and expansion destroys the surfactant material and new material is continually being discharged onto the alveolar surface. Thus, the reported high rate of pulmonary phospholipid synthesis is believed necessary for a rapid renewal of phospholipids in the surfactant

system (1, 2, 12, 26). The degree to which decreased lecithin turnover affects lecithin content and thereby alters properties of surfactant material requires further investigation. Such information may provide clues to the underlying mechanism causing the observed edematous and atelectatic conditions found after prolonged exposure to NO_2 (3, 6, 10, 25).

Summary. The effect of breathing nitrogen dioxide (NO_2) on the *in vivo* incorporation and turnover of ^{14}C-1-palmitate in rat lung lecithin was studied. Continuous exposure to 5 ppm of NO_2 for 14 days markedly decreased lecithin turnover. The suggestion is made that changes in lecithin turnover may lead to alterations in the surface-active material on the alveolar surface which in turn may account for some of the damage observed in NO_2-exposed lungs.

We thank Barbara Pyle for her technical assistance. The advice of Dr. Richard Scholz is gratefully acknowledged.

1. Abrams, M. E., J. Appl. Physiol. **21**, 718 (1966).

2. Abrams, M. E., and Wigglesworth, A., Biochem. J. **3**, 21 (1968).

3. Blair, W. H., Henry, M. C., and Ehrlich, R., Arch. Environ. Health **18**, 186 (1969).

4. Brown, E. S., Amer. J. Physiol. **207**, 402 (1964).

5. Clements, J. A., Hustead, R. F., Johnson, R. P., and Gribetz, I., J. Appl. Physiol. **16**, 444 (1961).

6. Davidson, J. T., Lillington, G. A., Hayden, G. B., and Wasserman, K., Amer. Rev. Resp. Dis. **95**, 790 (1967).

7. Felts, J. M., Med. Thorac. **22**, 89 (1965).

8. Folch, J., Lees, M., and Sloane-Stanley, G. H., J. Biol. Chem. **226**, 497 (1957).

9. Freeman, G., Crane, S. C., Stephens, R. S., and Furiosi, N. J., Amer. Rev. Resp. Dis. **98**, 429 (1968).

10. Freeman, G., Crane, S. C., Stephens, R. S., and Furiosi, N. J., Yale J. Biol. Med. **40**, 566 (1968).

11. Fujiwara, T. F., Adams, H., Sipos, and S., El-Salawy, A., Amer. J. Physiol. **215**, 375 (1968).

12. Galdstone, M., and Shah, D. O., Biochim. Biophys. Acta **137**, 255 (1967).

13. Gluck, L., Sribney, M., and Kulovich, M. V., Pediat. Res. **1**, 237 (1967).

14. Hayden, G. B., Davidson, J. T., Lillington, G. A., and Wasserman, K., Amer. Rev. Resp. Dis. **95**, 797 (1967).

15. Hinners, R. G., Burkart, S. K., and Punte, C. L., Arch. Environ. Health **16**, 194 (1968).

16. Michal, G., Naegle, S., Danforth, W., Ballard, F., and Bing, R. Amer. J. Physiol. **197**, 1147 (1959).

17. Morgan, T. E., Finley, T. N., and Fialkow, H., Biochim. Biophys. Acta **106**, 403 (1965).

18. Morrison, W. R., Anal. Biochem. **11**, 218 (1964).

19. Parsons, J. G., PhD thesis, The Pennsylvania State University, 1968.

20. Pattle, R. E., Physiol. Rev. **45**, 48 (1965).

21. Pattle, R. E., Arch. Environ. Health **14**, 70 (1967).

22. Saunders, A. P., Hale, D. M., and Miller, A. T., Amer. J. Physiol. **202**, 443 (1965).

23. Scarpelli, E. M., and Clutanio, B. C., J. Appl. Physiol. **23**, 880 (1965).

24. Scarpelli, E. M., "The Surfactant System of the Lung," 220 pp. Lea & Febiger: Philadelphia (1968).

25. Steadman, B. L., Jones, R. A., Rector, D. E., and Siegel, J., Toxicol. Appl. Pharmacol. **9**, 160 (1966).

26. Tierney, D. F., Clements, J. A., and Tranhan, H. S., Amer. J. Physiol. **213**, 671 (1967).

27. Zilversmit, D. B., Entenman, C., and Fishler, M. C., J. Gen. Physiol. **26**, 325 (1943).

Received May 8, 1970. P.S.E.B.M., 1970, Vol. 134.