drivers coming from work showed a mean COHb saturation of 6.9 percent with a range from 3.0 to 13.0 percent.

Large international airports, such as O'Hare and J.F.K., had surprisingly high ambient CO concentrations attributable in part to jet engine CO production and in part to the high automobile density near airport terminal entrances. Persons with advanced heart or lung disease planning to travel in aircraft pressurized for 6000 feet could, as a result of prolonged CO exposure in airport terminals, unknowingly subject themselves to an additional anoxic stress.

The major purpose of our investigation was to establish the range of CO exposure experienced by the American population in these four cities in 1969 to 1972. In so doing we have established that a significant percentage of their populations was continuously exposed to CO concentrations in excess of those permitted by the air quality standards. These worrisome baseline data should stimuate a reexamination of the scientific basis for the air quality standards and should provide a means to measure the effectiveness of the antipollution measures which we as a nation develop and use to control CO exposure.

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Linoleic Acid Hydroperoxide: Impaired Bacterial Uptake by Alveolar Macrophages, a Mechanism of Oxidant Lung Injury

Abstract. *Exogenous linoleic acid hydroperoxide causes in vitro impairment of both bacterial uptake and the phagocytic stimulation of $^{14}CO_2$ production from [1-^{14}C]glucose in rabbit alveolar macrophages by an undefined effect on the cell membrane. This effect may be one mechanism for the defective pulmonary bacterial clearance characteristic of oxidant lung injury.*

Exposure of lungs to oxidants (NO_2, O_3, and excess O_2) has many effects, including impaired pulmonary bactericidal activity (*1*) and the formation of lipid hydroperoxides (*2*). Although pulmonary bacterial clearance is a complex process, one important factor is bacterial ingestion by alveolar macrophages (AM). While lipid hydroperoxides are highly cytotoxic (*3*) and affect a number of membrane functions, notably in erythrocytes (*4*), their effect on phagocytosis has not been studied. We therefore studied the effects of exogenous linoleic acid hydroperoxide (LPO) on bacterial uptake and glucose metabolism in the AM.

The LPO was prepared by aerobic oxidation of linoleic acid and purified by silicic acid column chromatography.

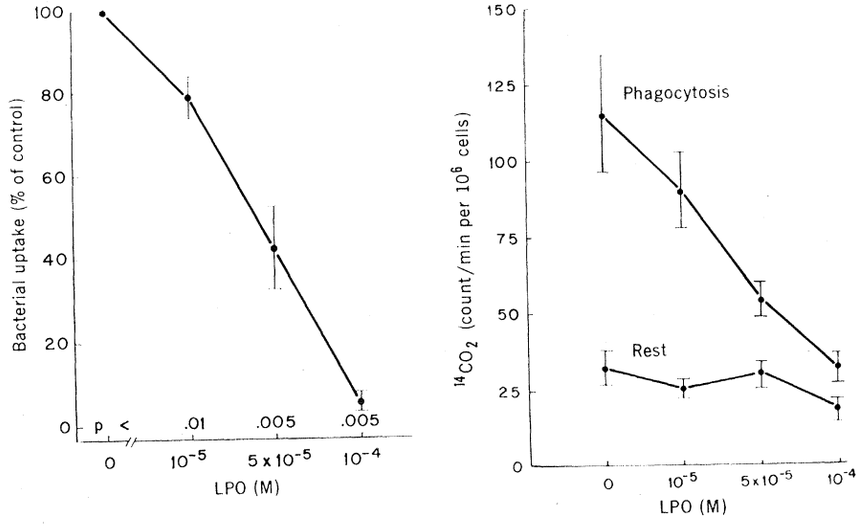

Fig. 1 (left). Effects of LPO on bacterial uptake. Alveolar macrophages (1×10^7) were suspended in 2 ml of GRPS (Ringer-phosphate solution containing 5.5 m*M* glucose, *p*H 7.4) and the appropriate amount of LPO. Control flasks contained no AM. After incubation at 37°C, for 1 hour, 0.25 ml of homologous serum and 2.5×10^8 live *Staphylococcus aureus* 502 A were added to both control and experimental flasks, and the incubation was continued for another hour. The AM were removed by centrifugation at 2500*g* for 15 minutes, and the supernatants containing bacteria were quantitatively cultured by a pour plate technique. Bacterial uptake was calculated from the difference between the colony counts in the absence and presence of AM. Light microscopy indicated that the majority of the bacteria are ingested by the AM, although some surface adherence cannot be excluded. Bacterial uptake in control flasks (no LPO) was taken as 100 percent. Data from seven experiments are expressed as percentage of control ± 1 standard error (S.E.). Fig. 2 (right). Effect of LPO on $^{14}CO_2$ production from [1-^{14}C]glucose. Alveolar macrophages (2×10^8) were suspended in 2 ml of GRPS in the presence of the appropriate amount of LPO. After 1 hour of incubation, 12.5 percent homologous serum, 0.1 μc of [1-^{14}C]glucose, and, where appropriate, 2×10^{10} heat-killed *Staphylococcus epidermidis* were added and the incubation continued for another hour. Production of $^{14}CO_2$ was measured as described (*8*). Data are mean ± 1 S.E. for five experiments performed in triplicate.

Its purity was confirmed by homogeneity on thin-layer chromatography and ultraviolet spectroscopy.

Bacterial uptake by AM was progressively impaired by increasing concentrations of LPO (Fig. 1): Uptake was inhibited 23 percent in the presence of $10^{-5}M$ LPO. Since bacterial uptake stimulates glucose conversion to CO_2 (*5*), we examined the effects of LPO on $^{14}CO_2$ production from [1-^{14}C]glucose by both resting and phagocytizing AM. These data (Fig. 2) indicate that increasing concentrations of LPO cause a similar progressive diminution in the $^{14}CO_2$ production associated with bacterial entry, notably a 25 percent inhibition with $10^{-5}M$ LPO. By contrast, the resting cells showed a small but insignificant inhibition (30 percent, $P > .1$) of $^{14}CO_2$ production in the presence of ten times as much LPO ($10^{-4}M$). With $10^{-4}M$ LPO, little bacterial entry occurs (90 percent inhibition) (Fig. 1), and there is a minimal stimulation of $^{14}CO_2$ production after bacteria are added (Fig. 2). We conclude that the major effects of these concentrations of LPO (10^{-5} and $10^{-4}M$) on $^{14}CO_2$ production from glucose by the AM in the presence of bacteria depend on an impairment of bacterial entry and are not a manifestation of general cytotoxicity. The latter inference is confirmed by the observation that cell viability was unaffected by $10^{-4}M$ LPO, as judged by the exclusion of eosin Y.

Lipid hydroperoxides inhibit sulfhydryl enzymes (*6*), so we examined the effects of LPO on three such dehydrogenases involved in glucose oxidation. These data (Fig. 3) show minimal impairment of these enzyme activities when intact AM are incubated for 1 hour in the presence of $10^{-4}M$ LPO, a concentration that drastically impaired bacterial entry (Fig. 1). These small effects on the enzyme activities do not explain the inhibition of bacterial uptake. Higher LPO concentrations caused progressive impairment of the activities of these three enzymes, notably affecting glyceraldehyde-3-phosphate dehydrogenase.

It is possible that LPO enters AM in a "piggyback" fashion during bacterial entry, hence the effects of $10^{-4}M$ LPO on these three enzymes in the presence of bacteria were examined. The presence of bacteria did not enhance the inhibition of these enzyme activities by LPO. Thus, the "piggyback" hypothesis seems unlikely. Furthermore, $10^{-5}M$ LPO caused complete inhibition of these three enzyme activities in AM disrupted by sonication. These last two experiments indicate that LPO does not enter the intact cell; therefore, its effect on bacterial entry probably depends on as yet uncharacterized alterations of the cell membrane.

Another aspect of AM metabolism is the fate of endogenous lipid hydroperoxides, whose formation is stimulated by phagocytosis (*7*). Rabbit AM contain vigorous glutathione peroxidase activity, measured with H_2O_2 as substrate (*8*). With LPO as substrate, glutathione peroxidase activity in sonicated AM was similarly measured. The activities with LPO and H_2O_2 were, respectively, 22.6 ± 1.8 and 20.0 ± 0.80 nmole of reduced nicotinamide adenine dinucleotide phosphate oxidized per minute per 10^6 AM. Glutathione peroxidase activity with LPO as substrate was also present in the supernatant after centrifugation of the sonicated material and was proportional to the number of AM in the assay; the activity was destroyed by boiling the AM sonicate. The activity was inhibited by the sulfhydryl binding agent, *N*-ethylmaleimide; at $5 \times 10^{-5}M$, this compound caused 50 percent inhibition with either LPO or H_2O_2 as substrate.

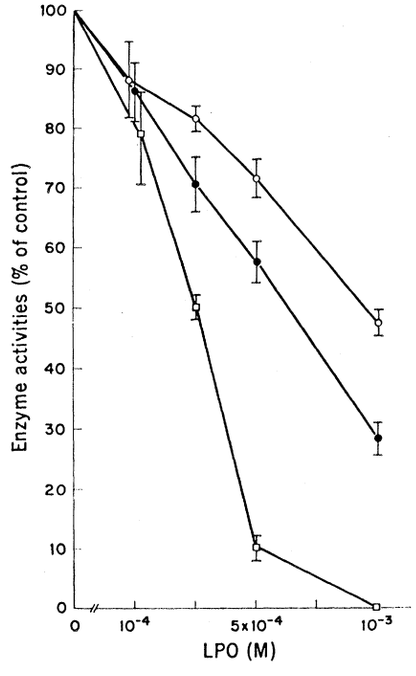

Fig. 3. The effect of LPO on three dehydrogenases, 6-phosphogluconate dehydrogenase (○), glucose-6-phosphate dehydrogenase (●), and glyceraldehyde-3-phosphate dehydrogenase (□). Intact AM (2×10^7 in 2 ml of GRPS) were incubated for 1 hour at 37°C, either alone (control) or with the indicated concentration of LPO. The AM were then centrifuged, washed in 2 ml of GRPS, sonicated, and centrifuged again. Enzyme activities in the supernatant after sonication were assayed as described [6-phosphogluconate dehydrogenase (*13*); glucose-6-phosphate dehydrogenase (*14*); glyceraldehyde-3-phosphate dehydrogenase (*15*)]. Control enzyme activities (no LPO) were taken as 100 percent. Data from seven experiments are expressed as percentage of control ± 1 S.E.

These data provide new evidence that LPO, one representative of a chemical species common to several types of oxidant lung injury, impaired a critical pulmonary defense mechanism present in the noninflamed lung —namely, microbial ingestion by the AM (*9*). In addition, AM contain a glutathione system (*8*) that can detoxify endogenous lipid peroxides. This system is also present in whole lung tissue, and its activity increases during chronic low exposure to oxidant (*10*). Further, the antioxidant effect of selenium (*11*) has been reported to depend on its presence in glutathione peroxidase (*12*).

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