Variation of the *p*H of the incubation medium showed optimum activity from *p*H 6.8 to 7.5. The system was inactive in the absence of reduced NADP (or a NADPH-generating system). Lack of oxygen abolished MEOS activity; reduction of the O_2 from 21 to 4 percent diminished MEOS activity by 45 percent ($P < .01$). Carbon monoxide reduced MEOS activity even further, approximately 67 percent ($P < .001$). Sodium cyanide (0.1 m*M*) inhibited MEOS activity by 17 percent.

Feeding of ethanol resulted in a significant increase in the activity of hepatic MEOS; it averaged 7.70 ± 1.02 units in the nine male rats fed the control diets for 24 days, whereas in the littermates pair-fed daily with isocaloric amounts of the alcohol-containing diet, the activity was 9.95 ± 0.87 units ($P < .05$). A greater increase was observed in the female rats: 5.0 ± 0.59 units in the controls versus 11.1 ± 1.45 units after ethanol ($P < .01$). Contrasting with MEOS, ADH activity was unaffected by the alcohol feeding.

The results of the present investigation show that microsomes of liver tissue contain an active ethanol-oxidizing system. Preliminary results also indicate that a similar system exists in human liver (*12*). Hitherto, ADH was generally thought to be the only enzyme responsible for the oxidation of ethanol in vivo (*13*), though in vitro, catalase is also active (*5*). That MEOS activity is different from ADH is indicated by (i) the cofactor requirements (NAD for ADH and NADPH for MEOS); (ii) the effect of *p*H, the optimum *p*H for the oxidation of ethanol to acetaldehyde by ADH being 10.8 (*5*), whereas the optimum for MEOS is physiological *p*H. Consequently, under our standard conditions for measuring MEOS activity, purified ADH was inactive. That catalase is not likely to be responsible for MEOS activity is indicated by its distribution. Most of the hepatic catalase is localized in the cytosol and "mitochondria" (*14*). These fractions had negligible ethanol-oxidizing activity under our assay conditions for MEOS measurement. Moreover, cyanide only partially inhibited MEOS activity at a concentration (0.1 m*M*) which almost completely abolishes catalase activity (*15*).

Characteristics of MEOS (requirement for O_2, NADPH, and partial inhibition by CO) resemble those commonly found among microsomal drug-detoxifying enzymes (*4*), but differ from the microsomal system reported to oxidize primarily methanol and to be insensitive to CO (*16*). Like that for other drug-detoxifying enzymes, the MEOS activity increased significantly after induction by substrate administration. Though MEOS activity in control rats was lower in females than in males, the capacity for adaptation was greater in the females, a sex difference common for microsomal drug-detoxifying enzymes (*4*).

Though reports by others concerning the possibility of ADH adaptation have been conflicting (*17*), our observation of a lack of ADH adaptation is in accord with several previous publications (*18*). Furthermore, whereas ADH is found in the cytosol (*6*), a number of ethanol effects involve the microsomes (*17*). Though the quantitative role of the microsomal ethanol-oxidizing system (MEOS) in vivo, remains unknown, demonstration of the existence of MEOS and its adaptive increase helps to explain a number of poorly understood effects of ethanol (*17*).

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Phagocytosis of Inhaled Plutonium Oxide–^{239}Pu Particles by Pulmonary Macrophages

Abstract. *Pulmonary macrophages and plutonium particles were removed by washing the lungs of rats that had inhaled plutonium oxide–^{239}Pu. A significant amount of plutonium was found in multiple washings of the same lung. The removal of toxic particles by washing is of potential therapeutic value. Particles were phagocytized by macrophages during the first 3 hours and retained within these cells for up to 25 days. Nearly all particles in washings were found in macrophages after the second day. The percent of macrophages with engulfed particles increased with increasing amounts of plutonium deposited in the lungs. The ability of pulmonary macrophages to rapidly phagocytize and retain plutonium particles deposited in the lungs has been shown.*

The role of the pulmonary macrophage in the clearance of plutonium particles deposited in alveoli has remained somewhat speculative. The deposition, retention, translocation, and excretion of inhaled $^{239}PuO_2$ particles has been previously described (*1–4*). Studies of the rat peritoneal cavity demonstrated the ability of mononuclear phagocytes to rapidly phagocytize and accumulate plutonium particles (*5*, *6*). Studies were initiated in order to define the role of the pulmonary macrophage in determin-

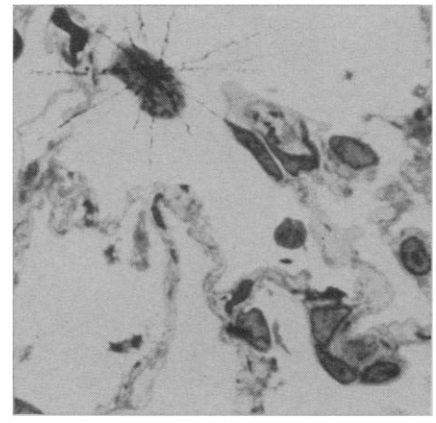
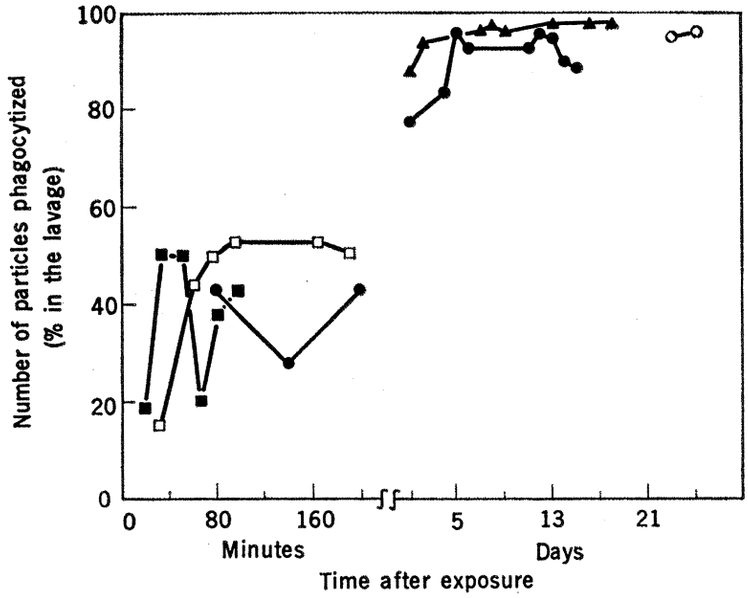


Fig. 1. Phagocytosis of plutonium particles by pulmonary macrophages. Section of unworked lung removed at 7 days after inhalation, fixed in glutaraldehyde, and embedded in epon. Autoradiogram, 14-day exposure, Richardson's stain (*10*). Note alveolar macrophage with alpha tracks from phagocytized plutonium particle(s) in upper left-hand corner within an alveolus. A similar particle distribution is seen on autoradiograms of cytosmears from lung washings. (×975)



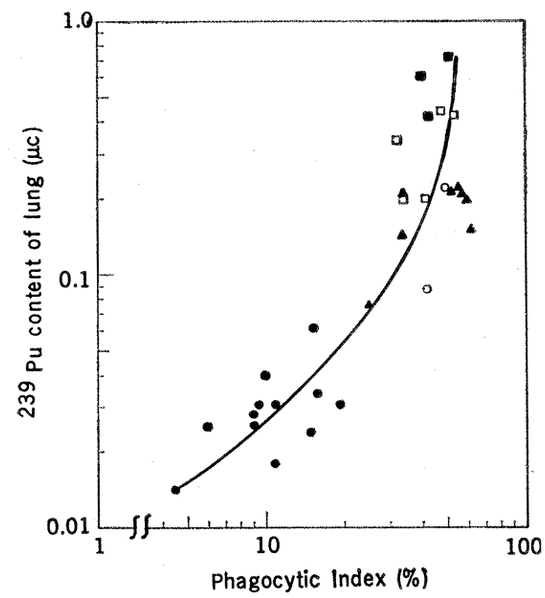


Fig. 2 (left). The percentage of plutonium particles found in the first lung lavage which had been phagocytized by pulmonary macrophages, at intervals following inhalation. Each point represents an individual measurement. About 50 percent of the initially deposited plutonium was cleared from the lung during the first few hours. The range of terminal amounts of plutonium (μc) in the lungs of each group, and the symbols for the groups are as follows: ●, .014 to .062; □, .198 to .437; ■, .340 to .725; ○, .085 to .514; ▲, .077 to .552. Fig. 3 (right). Correlation between the amount of plutonium present in the lung after one lung washing and the phagocytic index. Each point represents an individual measurement taken from 1 hour to 25 days after inhalation of particles.

ing the early distribution and fate of inhaled plutonium particles. The possible efficacy of pulmonary washing for the removal of inhaled particles was a tangential observation of this study.

Fifty-four Sprague-Dawley female rats (240 to 300 g body weight) were exposed to aerosols of $^{239}PuO_2$ particles with count mean diameters ranging from 0.1 to 0.2 μ. Less than 0.5 percent of ^{239}Pu was in the ionic state, as determined by ultracentrifugation and dialysis. The production of aerosols and a description of exposure equipment and procedures have been described (*3*). At 15 minutes to 25 days, after a 5- to 15-minute aerosol exposure, rats were lightly anesthetized with ether and exsanguinated. The trachea was isolated and clamped and the lungs were removed and washed with 12 ml of saline. The wash was introduced with a syringe into the trachea, resulting in a two- to threefold expansion of the lung volume, and then collected by inverting the lungs over a beaker. Cells in the wash were isolated by centrifugation and resuspended in normal rat serums, and smears were prepared. For autoradiography, slides were coated with an Ilford K-5 emulsion, about 10 μ thick, and exposed for 14 days. The percentage of particles which had been phagocytized by pulmonary macrophages, and the phagocytic index, that is, the percent of pulmonary macrophages with engulfed particles (*7*), were determined from autoradiograms. Two or more alpha tracks in the autoradiographic emulsion, originating from the same point, were considered as identifying a particle. Positive intracellular identification of plutonium particles in phagocytes was previously accomplished by electron microscopic autoradiography (*8*). The plutonium contents of washed lungs and selected lung washes were determined by dissolving samples in acid and counting in a liquid scintillation system.

To determine the amount of phagocytosis occurring in centrifuge tubes during isolation of cells and particles from washes, 0.1 μc of $^{239}PuO_2$ particles were added to lung washings from three unexposed rats, and the cells were isolated for autoradiographic examination. Less than 4 percent of the particles were phagocytized, and less than 5 percent of the macrophages had engulfed particles, indicating minimal, in vitro, phagocytosis of plutonium particles during the time required to prepare the smears.

The lungs of killed rats contained from 0.07 to 0.7 μc of plutonium. A maximum of 18 percent of the total lung plutonium was removed with a saline wash, 20 minutes after exposure, as compared to 1 to 6 percent removed after 25 days (data from washings of 18 rats). From 30 to 40 percent of lung plutonium was removed by five successive, 12-ml saline washings during the first 7 days after exposure (data from washings of five rats). Fifty percent of lung plutonium was removed from one rat at 16 days by ten 12-ml saline washings of the lungs. Bronchopulmonary lavage is used in the treatment of pulmonary alveolar proteinosis (*9*). Such lavage techniques may be of potential therapeutic value in the removal of inhaled radioactive materials.

The intracellular localization of plutonium particles within pulmonary macrophages was demonstrated by autoradiography of smears and lung sections (Fig. 1). From 20 to 50 percent of all plutonium particles found in the first wash were phagocytized by pulmonary macrophages during the first 3 hours; over 80 percent, and usually over 95 percent, of these particles were found within macrophages after the first day (Fig. 2). The rate of phagocytosis of plutonium particles in the lungs during the first 3 hours was similar to that observed in peritoneal mononuclear phagocytes (*6*). Those particles falling on the mucus blanket lining the trachea, bronchi, and bronchioles are mostly cleared from the lungs and excreted in the feces during the first few days (*3*). About 50 percent of the initially deposited plutonium was cleared from the lungs during the first few hours. Less than 10 percent of the 24-hour lung burden was cleared during the next 24 days. Only about half of the particles on smears of lung washings taken during the first few hours had been phagocytized (Fig. 2). The particles which were not phago-

cytized may have been initially deposited on the mucus lining of the trachea, bronchi, and bronchioles and cleared during the first day.

The percentage of pulmonary macrophages with engulfed particles increased to a maximum value during the first 2 hours. From 5 to 65 percent of the isolated pulmonary macrophages had phagocytized particles during the first 3 hours. The phagocytic indices of each group were essentially unchanged during the succeeding 25-day observation period, although considerable variation among individuals occurred. The phagocytic index rose with increasing amount of plutonium deposited in the lungs (Fig. 3), demonstrating that the number of particles phagocytized was somewhat dependent on the number of particles that were administered and deposited in the lungs.

These preliminary results showed that a large number of the plutonium particles deposited in lung, and subject to a slow clearance with a biological half-life of several hundred days (*1*, *3*), were phagocytized and retained within pulmonary macrophages during the 25-day observation period. Death of macrophages and rephagocytosis of particles may have occurred during this period. The technique of pulmonary washing appears to offer an experimental method for the quantitative study of the deposition and retention of inhaled particles as well as offering possibilities for therapeutic removal of toxic particles from the lungs of accidentally exposed individuals.

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Cognitive Capacities of Young Children: A Replication

Abstract. *Children between the ages of 3 years and 4 years 7 months correctly respond to the addition of objects in an array, to the numerical equality of arrays, and to their relative numbers. However, they are not able to conceptualize equality or inequality when objects are misaligned or spatially transformed. The Mehler and Bever assertion that very young children conserve the concept of number, with a decline and rise in performance, is not confirmed.*

Mehler and Bever (*1*) have reported that children below the age of 4 are capable of conserving concepts of quantity. This finding contradicts the reports of Piaget (*2*) and others who place these acquisitions at about age 6 to 7. This age difference in the acquisition of conservation is of theoretical consequence. Mehler and Bever's delineation of a decline in conservation performance from the very youngest age studied (2 years 4 months) and then a rise (with age) implies that these capacities are genetically determined and are a "basic characteristic of man's native endowment" (*1*). The Piaget position, on the other hand, is that these competencies reflect the influence of maturational and experiential determinants under the control of an internal self-regulating mechanism.

In contrast to the usual procedure of testing conservation with a relocation or transformation of objects first identified as equal with respect to a quantitative property, Mehler and Bever used a conservation-of-inequality task based on the inequality of two sets of objects. More importantly, they both added objects to their numerical arrays and relocated them in a single operation. Thus it is not possible to know whether a child's response was due to addition or relocation, or both. It was my prediction that a controlled study which separated these operations would show that the results were due solely to the child's ability to deal with "more" in the additive sense of "more of," or in the relational sense of "more than," rather than with the conservation concept of "more than in the face of a transformation." I now report results of an experiment designed to test the correctness of this prediction.

The concepts measured were as follows: (i) Conservation of equality based upon an initial response of "same" to equal numbers of M & M's (candy-coated chocolate pellets) set in two rows and in one-to-one correspondence (the "before" condition of Fig. 1), followed by a relocation of one row either by expansion or contraction, and a continued response of "same" to the numbers in the two rows (the "after" condition). (ii) Conservation of inequality in which the "before transformation" condition is one of inequality of both number and location; the "after" condition is still one of inequality, but the beginning and terminal points of the rows are in either one-to-one correspondence or in a different spatial location from the "before" condition. In earlier studies (*3*, *4*) a distinction was made between transformational and static conservation. In the latter case no transformation takes place in the stimulus. A conserving subject is required to judge two rows as numerically equal in spite of a lack of alignment between them. In my study both static and transformation-conservation tasks were included in the test. (iii) Relational concept ("more than"): two rows unequal in number and extension had to be judged as unequal in number. (iv) Equality: two rows, equal in both number and extension (that is, in one-to-one correspondence) had to be judged as equal in number. (v) Additive concepts ("more of" or "less of"): one or two rows were presented and M & M's were added to or subtracted from a row.

In each trial a Mommy or Daddy doll accompanied each row. Responses represented a choice between the "same" or "different" and "more" or "same" amounts to eat. Two groups of children were tested in private nursery schools. One group was given the transformation tasks and the other the static tasks. Both groups were administered the same additivity tasks.

The data in Table 1 show different levels of performance. The level of conservation performance for conditions