TOXICOLOGY: THE RESPIRATORY TRACT[1] 6521

Louis J. Casarett
Pharmacology Department, University of Hawaii, Leahi Hospital, Honolulu, Hawaii

A heightened intensity of research in pulmonary toxicology in recent years has derived from several factors. One of the most compelling factors is the wide dissemination of environmental toxicants which produce pulmonary effects or enter via the respiratory tract. The massive volumes of scientific, technical, and lay literature are telling testimony to the general concern about the effects of environmental contamination on the biosphere and specific concern about airborne toxicants and human health.

Organic materials that are subject to volatilization and are relevant to the respiratory tract have been reviewed among others, by Gerarde (1), and Fassett (2). Reviews on inorganic or radioactive materials appearing in the pharmacological literature (3, 4) give some recognition to the complexities of the respiratory route of entry. A review by Foreman (5) should be singled out as emphasizing the airborne inorganic materials. Despite the lapse of some years, these reviews provide a base for understanding much of what is known about important sources of occupational exposures to organic materials, metals, and minerals. The subject of air pollutants, despite a full measure of attention, warrants some treatment if only because of the immediacy of the societal problems to which they contribute. Selective facets of air pollutant exposures and effects have been singled out for discussion as they have not received adequate coverage in the pharmacological literature and deserve special attention.

Deposition and clearance of particles, the alveolar response, and early lung changes have been subjects for review. Selected sources of general interest reflect a wide range of focus and format. One finds discourses on respirable air sampling such as that of Lippman (6), monographs on deposition and retention of particulate material, e.g., Hatch & Gross (7), and specific reviews devoted to factors controlling deposition (8) and retention (9).

Growing interest in mixtures of toxicants is manifest in numerous symposia. Some have been devoted solely to early events in the lung following inhalation (10, 11) or to specific topics such as air pollution and lung bio-

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chemistry (12). A major effort in recent years has produced predictive models for pulmonary deposition and clearance (13). Although derived from consideration of radioactive materials, such models have a clear and sometimes neglected applicability to chemical toxicants and, incidentally, to therapeutic agents for which a respiratory route of administration is used or contemplated. Some of these facets have been included with a view of alveolar clearance mechanisms in a recent essay (14).

Dose and Human Health

As pointed out by Stokinger (15), data on the constitution of the atmosphere to which man is exposed is and will continue to be necessary. They are of special import in epidemiologic studies of disease patterns in different geographic locations. It was also predicted (15) that small concentrations of more potent toxicants would be found as part of our atmosphere. These admonitions, which still hold true, implicitly underline the need for measurement of dose to the respiratory tract. Efforts expended in simple data gathering on atmospheric constituents do not usually result in a useful estimate of pulmonary dose of either the individual chemicals or the mixture. It is virtually axiomatic, in addition, that each collection of data will be different if all the components are measured.

Furthermore, atmospheric sampling does not measure the full pulmonary toxicant dose. The ingredients of pulmonary toxicology in the general population are many and the major focus is usually on aspects related to what has been called "civilization illness" by Pirket (16). That a significant part of the contribution to environmental pollution is from industrial sources is indisputable and measurable as a potential source of pulmonary dose although the contribution of natural pollution by the biosphere is often ignored (17). One also is readily convinced that public contribution such as automotive exhaust is both significant and measurable en masse. Even such a volitional exposure as smoking is subject to an approximation of dose.

Other sources of pulmonary toxicants are not readily subject to detection or to estimation of dose. For example the demonstrable presence of carcinogens in charcoal grilling of meats (18) is virtually impossible to quantitate in terms of true population exposure; combustion of plant and mineral material inside or outside homes represents another variable source of pulmonary exposure; the myriad chemicals available for home use are not readily subject to accurate assessment. A perspective on dose would suggest, for example, that the presence of an organic solvent in the atmosphere at mean levels of parts per billion or per trillion, is of distinct interest as part of the chemical mixture available to the respiratory tract. If other sources yield exposures higher by factors of thousands to millions to identical or similar solvents and to variable numbers of individuals in the same population, clearly the relation of disease to such atmospheric data is of diminishing significance.

A complex situation is further confounded by many other factors related

both to dose and to response. Brief allusion only may be made here to four —synergism, antagonism, adaptation or tolerance, and genetic propensity. There is no question that pulmonary toxicologists have much to study on episodic events of acute or subacute intoxication and exacerbation of existing disease states by air pollutants. Stokinger however, points out (19) quite correctly, that with a few possible exceptions, there are no unequivocally demonstrated chronic effects of pollutants on human health at levels usually in existence in our environment. What eludes us at present is the application of principles underlying clear but fragmentary examples of synergism to the assessment of effects of pollution on populations. Components of the atmosphere are known to produce similar or additive effects and it is inconceivable that some effect, however subtle, will fail of discovery.

Masking of synergistic effects may occur by direct or indirect antagonism. A timely discussion of this topic has appeared (19) in which several examples are cited. Some are direct and experimentally verified such as the antagonism between As and Se. Reference is also made to exciting studies of the competition among structurally similar carcinogens by Falk et al (20). One may see, in epidemiologic studies, a different level of "antagonism," such as the "protective" action against lung cancer suggested by exposure to coal and SO_2 (21), perhaps through the maintenance of more active cellular defense mechanisms.

A third element that confuses the population dose-response relation is the development of tolerance or the adaptation of the individual member of the population. An excellent review on adaptation to air pollutants has been presented by Morrow (22) and the phenomenon has been alluded to by Stokinger (19). An increasing number of references to adaptation are appearing and one may expect the assumption of a greater significance and recognition of the process in the assessment of effects of air pollution on human health.

High expectation exists also for practical recognition of examples of the fourth selected variable relating to population dose-response studies, viz, genetic propensity or susceptibility to diseases. There are numerous examples of metabolic diseases which are genetically determined, and variability of response to drugs is often explicable on the basis of metabolic capability, but only a few examples of diseases related to possible effects of air pollutants have come to light. The most relevant and most significant of these is alpha-1 antitrypsin deficiency and the increased susceptibility to pulmonary emphysema reported by Eriksson (23). This and other indices of susceptibility to disease or enhanced responses to toxicants have been discussed by Stokinger (19, 24).

Pollutant Gases

Carbon monoxide.—Despite considerable clinical experience and the elucidation of the basic mechanism of CO action, some questions about this compound remain. One of these concerns the persistence or residual effect

of acute intoxications. A recent clinical study of 206 cases summarizes pertinent previous clinical data (25). This work and others (26) have emphasized an apparent cerebral dysfunction following recovery from CO poisoning, a dysfunction that may persist for some weeks. The salutary effect of atmospheric or hyperbaric oxygen may well be of importance not only in enhancing the reversal of carboxyhemoglobin but also may serve to protect against a residual effect on brain.

Persistence of effects is one basis for suspicion that there may be a chronic toxicity for CO. A number of other measures show some persistence both in acute poisoning, and in experimental exposures at lower doses. For example, the hyperglycemia reported by Bour et al (27) as a common occurrence in acute poisoning has been demonstrated in rabbits with short exposures to 100–300 ppm (28). Hyperglycemia was found to persist beyond the period of exposure. Similarly a rise of fibrinolytic activity has been shown by El-Attar (29) in acute poisonings and in individuals repeatedly exposed. The increased activity was marked in the poisoning cases and rose higher and was more consistently elevated in those repetitively exposed to CO.

Other indications of a possible chronic toxicity of CO may be found in the biochemical behavior and degradation of CO (30) and in the direct ultrastructural effects of CO on alveolar cells, a particularly significant finding by Niden (31). Chronic toxicity may be masked by several factors. One of these is adaptation which is clearly seen in the smoker who carries levels of carboxyhemoglobin asymptomatically compared with the same levels in a nonsmoker. In smoking and in exposure to an urban atmosphere the CO is only one element in a complex mixture, another difficulty in assessing chronic toxicity. As there is no clear evidence that unequivocally confirms or denies chronic toxicity of CO, the question remains moot.

Nitrogen dioxide.—Research on the oxides of nitrogen to which the public is exposed has been rapidly accelerated in recent years. An incomplete spectrum of studies includes acute and chronic exposures (32, 33) including studies at threshold limit values (34), effects on lung antibodies (35), on resistance to disease (36), on macrophages (37), and on enzymes (38).

Mechanistically, the effects of NO_2 in the lung are likely to be initiated by the "peroxidation" of lipids (Thomas et al 39), at points of unsaturation analogous to the effect of NO_2 on phospholipids of E. coli (40). The development of a laboratory animal model for the study of NO_2-induced emphysema (41) has been an important development in the continued investigation of basic pulmonary changes in response to oxides of nitrogen. Among other implications of this model it is clear that effects of NO_2 result in effects on other functions such as the capability to handle particulate material (42). Of particular toxicological significance in assessing residual effects from acute exposures or development of disease under chronic expo-

sures is the "healing process" after subacute exposures. Among the few groups which have highlighted this important area is that of Freeman and associates (43).

Focus on the direct cellular effects of NO_2 has overshadowed other areas of potential import. One example will be given. The bronchoconstriction by irritant gases and by particulate material is well recognized. This response may be considered to be causally or casually related to the mast cell degranulation which occurs in response to NO_2 (44). What effects the released contents of mast cells may have on the NO_2-induced cellular effects is unknown. Further, in addition to influence on alveolar clearance of particles by NO_2 (43), particulate material itself is capable of degranulating mast cells (45). The interrelations of these several components of effects are of particular importance in the inhalation of mixed atmospheres.

Ozone.—The literature on ozone has been reviewed so extensively (46, 47) and so recently (48) that only a few comments are in order. Menzell (48) has compared the action of ozone with oxygen toxicity and radiation. Particular attention has been directed to a free radical mediation of effects which is analogous to the "peroxidation" of lipids noted for NO_2. By further analogy to O_2 toxicity, at least part of the ozone effect may be expected to result from action on surfactant material (49). A component of the increase in susceptibility to infection following exposure to irritant gases probably relates to action at double bonds in the membrane of the alveolar macrophage (50) in a manner similar to that described for the effect on the red blood cell (51), a bonafide systemic effect of ozone.

Sulfur dioxide.—As with other pollutant gases, SO_2 has been studied extensively as a single irritant gas. Dominant aspects of research in the past have included studies of absorption (52), effects of acute (53), chronic (54), and periodic (55) exposures and ventilatory changes in animals (53, 56, 57) and man (58). With SO_2 more than with other gases, fundamental research has been carried out for some years with a combination of the gas and aerosols (56, 58–61). Some aspects of this work have been reviewed (62) and Amdur has reported on the toxicology of decay products of SO_2 (63) which presage additional critical studies of combined effects of gaseous agents and aerosols.

Pulmonary Effects

Several allusions to effects of pollutant gases have been made in the brief review presented above. Some aspects of effects have been singled out for emphasis. One of these is the study of the development of pulmonary dysfunction and progressive pathologic change from inhaled toxicants, particularly emphysema, which has been hampered by disagreement about the applicability of animal responses to human disease. A second area of em-

phasis is the influence of pollutant gases on susceptibility to infection and the functional potency of the alveolar macrophage. A third, and important, aspect concerns the noncellular components of lung tissue, particularly "surfactant."

Emphysema.—Some aspects of oxidant-induced cellular and biochemical changes related to emphysematous changes and other effects have recently been reviewed briefly by Heuter (64). In addition to the model proposed by Haydon et al (41), papain injection has also been used. The most recent structural and biochemical study of papain-induced emphysema in hamsters is that of Kilburn & Dowell (65). With papain, these investigators have shown a pattern of initial change in endothelium followed by fluid and cellular infiltration of alveoli and loss of connective tissue as a baseline for subsequent superimposition of oxidant gases. The same investigators have examined structural and biochemical effects in dogs and rabbits after exposure to 5–16 ppm of NO_2 (66). Again, it is concluded that the major effect is on the endothelium and that this observation helps "to clarify the pathogenesis of pulmonary edema in animals exposed to oxidant gases."

The picture for papain however appears not to be entirely consonant with other observations. The use of NO_2 itself to elicit pulmonary changes in rat lung has been shown by Freeman and associates (43, 67, 68) to increase connective tissue rather than decrease it. It is particularly noteworthy that these changes occurred in the absence of edema or cell destruction on chronic exposure and during "healing." Thus although papain may produce at some point in time, a microscopic appearance resembling centrilobular emphysema, there is some question whether it is etiologically precise or realistic. Despite an interim value for papain it would appear that use of a proteolytic agent is not the most appropriate means for studying emphysema, especially that presumed to be caused by pollutant oxidants.

With emphysema or other pulmonary diseases, oxidants are usually lumped together in such a way as to imply that the development of the diseases have the same etiology and course of development. For example Huber et al (69), using oxygen and ozone, imply that these are identical in effects with other "oxidants." As a working experimental base such a view is certainly appropriate or as part of a stated goal as an "indicator of low level oxidant toxicity" (70). Further, one can recognize the oxidation of double bonds by several of the pollutant gases as a primary response of a chemical class; excellent evidence for protection by antoxidants has been provided by Roehm et al (71).

The complex events that occur as sequelae may however represent quite divergent effects. One example will serve to illustrate. Some apparent paradoxes in responses to oxidants have been attributed to actions on surface-active material of the lung. The cellular constitution of the alveolus, including cells which may produce surfactant, is of at least equal importance. A clear difference in cellular response has been demonstrated by Evans et al

(72) among gases that oxidize double bonds. While high concentrations of ozone (73) or oxygen (74) produced an inhibition of "cell renewal" as measured by uptake of tritiated thymidine, exposure to 17 ppm of NO_2 produced a significant increase in this index of cell turnover. That this was an initial response only is indicated by the return of the labeling index to normal at seven days, i.e., it was not sustained by continuous exposure. It is felt to be of more than academic interest that the time course of this effect is reminiscent of the alveolar response to Freund's adjuvant and to particulate Fe_2O_3 (75, 76) measured by the same index.

Susceptibility to infection.—The defense against infection is one of the more prominent roles of the lung subject to change by inhaled pollutants. Rylander (77) has presented a review of effects of air pollutants along with original data illustrating a decreased ciliary clearance of organisms from guinea pig lung after ethanol and cigarette smoke and a decreased inactivation of organisms by phagocytes after coal dust.

From a formidable literature on the clearance of microorganisms from the lung, a few examples of recent studies may be cited. Two particularly active groups led by Laurenzi and by Green have reported quantitative techniques for measuring the presence and viability of organisms in lung (78, 79), studies of clearance of bacteria from parenchyma of the lung via the alveolar macrophage (80, 81), and discussions of the mechanisms of clearance as related to infection (82, 83). In addition to the effects of ethanol (83) on clearance of bacteria, a phenomenon clearly demonstrated some 50 years ago, other variables have been shown, directly or inferentially, to affect the persistence of bacteria in the lung. Hypoxia has been shown to increase the rate of inactivation of bacteria (84) and to increase resistance to infection (85). Both adaptation to effects (85) and the lack of adaptation (84) were reported. Cold (84, 86) and to lesser extent, starvation and corticosteroids (83), reduce bacterial inactivation. Even nonpulmonary diseases such as acute renal failure have been shown to influence susceptibility to infection via the respiratory tract (87). The species and strain of organism have been recognized as a determinant in the rate of destruction of bacteria since the turn of the century and have also been demonstrated more recently (84, 88).

In retrospect, studies in the early part of the century were suggestive that irritants affect defense mechanisms against bacterial invasion. An increase in mortality from a streptococcal infection has been shown with ozone alone (89, 90) and with the added stress of cold (86). Ehrlich has compared results with NO_2 at 25 ppm for 2 hours (90) or 0.5 ppm for 6 months (36) with those for 0_3. Ozone exposure a full day after administration of the test organism increased mortality. Purvis et al (91) using the same concentration of ozone (4 ppm) administered ozone prior to the bacteria and found an increased infection at short intervals but no effect when the gas was given 19 hours before the organism. Although the mechanism

by which irritants increase susceptibility to bacteria may be partly related to effects on ciliary clearance (77, 80, 91) this effect must be viewed in conjunction with changes in function of alveolar macrophages, both the engulfment by macrophages and the subsequent lytic action of the cell being necessary for full inactivation (77).

The phagocytic process and related studies of the alveolar macrophages, some aspects of which have been reviewed recently (14), are too extensive for inclusion. Clearly some studies cited above reflect both a decreased phagocytosis of organisms and a retention of viability of the organism. In addition to direct action of pollutants on phagocytic cells, evidence has been presented for an effect of ozone on a "protective factor" for alveolar macrophages (92). This contention receives partial support from the simple finding that removal of fluid collected with cells in pulmonary lavage decreases the phagocytosis of inorganic particles (93). Even if engulfment occurs, bactericidal activity may be decreased by irritants such as ozone (94); examples of the most recent demonstrations of effects of oxidants on lytic enzymes of alveolar macrophages are the works of Gee et al (95) and Hurst & Coffin (96).

Just as hindsight permits "prediction" of effects of oxidants on infection liability, so does it allow for the effects of particles. Silica has long been recognized as enhancing tuberculosis susceptibility (97) and has been shown more recently to increase susceptibility to other organisms (98). This finding is consonant with cytotoxicity of silica, particularly its effect on cellular lytic enzymes as recently discussed by Allison (99). Although some early studies have demonstrated no effect of silica or even an enhanced resistance, the test organism used was usually the pneumococcus, which is not as readily phagocytized as other organisms (100). In studies during the last 70 years, there is ample evidence for an increased susceptibility to infection from overwhelming concentrations of relatively inert dusts and decreased susceptibility from small concentrations of dusts. The latter phenomenon may be explained by the increase in numbers and activity of cells in the alveolar regions in response to dust inhalation (76, 101).

Noncellular material.—Increasing attention has been given to the chemical constitution of lung (102), noncellular components of structure (102, 103), and the lining layer of lung alveoli, recently reviewed by Brooks (104). Certainly the most intriguing component of lung is the complex of materials which is customarily called "surfactant" and is thought to form a complete layer over the alveolar surface. From the early postulation of a low surface tension fluid in the lung by von Neergaard (105) and the empirical demonstration of a surface-active agent in edema fluid by Pattle (106) consideration of this material has been included in all aspects of pulmonary physiology, toxicology, and disease. A pertinent review of the subject is that of Heinemann (107).

Leaders in surfactant research have been Clements and colleagues who have made many contributions to the identification of main components of

surface-active material of the lung and to concepts of the physiological significance of surfactant (108–110). Most recently a set of comparative data on the constitution of surfactant in a variety of species has appeared (111). It is pointed out that dipalmitoyl phosphatidylcholine (dipalmitoyl-lecithin, DPL), a major ingredient of surfactant, appears to be present in quantity sufficient both to cover the surface of the respiratory tissue and provide a degree of reserve material. The synthetic pathway of greatest importance qualitatively is suggested to be from 1-α glycerophosphate and cytidine diphosphocholine (CDP-choline) (112). The turnover of surfactant lecithin has been studied in excised lungs (113) and in intact rats (114). Spitzer & Norman (115) have suggested that surfactant lecithin synthesized by way of the CDP-choline pathway is bound to protein prior to secretion into the alveolus as a molecular unit. Of particular interest is the synthesis of surfactant in relation to the development of the lung. On the one hand, prior to the development of surfactant in the fetus, such impinging conditions as hypoxia may decrease the synthesis of lecithin and be related to neonatal respiratory disease (116). On the other hand, as pointed out by Clements (110) the newborn, having initiated a high rate of synthesis, has a high reserve of surfactant. That this feature may be influenced in the opposite direction than that found for hypoxia has been shown by Knelson (117) who reported that administered steroids resulted both in an enhanced production of surfactant and in an earlier onset of the synthesis of surfactant in utero.

Pollutant mixtures.—Some studies of mixtures of gases have been carried out, for example O_3 with NO_2 (118) and SO_2 with NO_2 (119). Most often the effects of mixtures appear to be simply additive or substitutive (120). A strong trend toward realistic pollutant exposure is readily detectable in the literature. A review by Heiman (121) cites a number of animal studies of naturally occurring atmospheres through 1966. Similar studies have been carried out since that time. Despite the obvious realism of exposure to "smogs" existing in urban areas, a note of caution is in order. Even in rare circumstances when the constitution of the atmosphere is well characterized one is faced with the improbable task of selecting the degree to which each component contributes to observed effects. Atmospheres certainly vary from one location to another; in chronic studies the constituents may vary considerably from time to time in the same location. It is suggested that a degree of realism may fruitfully be abandoned for controlled studies of mixtures of compounds approximating real atmospheres and a comparison with effects noted from exposure to the individual components.

An especially important component of realistic atmospheres is particulate material. The pioneer work of Amdur with aerosols and SO_2 is of paramount importance from at least two points of view. First, particulate material is always present in the atmosphere and its role in the effects is therefore accountable in inhalation toxicology studies if the studies are to be interpreted in relation to the real environmental circumstances. Second, the

presence of particulate nuclei may markedly alter the total dose and the distribution of the dose of a gas in the respiratory tract. Both initial physiologic responses and, in chronic exposures, the ultimate pathologic events may be altered. The increased effects of SO_2 in deep pulmonary structures when given to guinea pigs with NaCl particles (59), although not as clearly demonstrable under experimental conditions in humans (60), still serves as the most plausible interpretation of differences of effects of SO_2 with and without dust in occupationally exposed people (122).

Particulate material, as a part of the mixture to which people are exposed, may also absorb vapors and alter the dose to the respiratory tract. For example, Schlipköter (123) has demonstrated an increased residence time in the rat lung for benzpyrene, a known carcinogen, when it was administered with carbon particles. A simple calculation of the surface area of a finely divided particle population readily illustrates that the deposition of an adsorbed gas or vapor—the dose—can be significantly greater than that occurring with an exposure without particles. Obversely, the gases or vapors may affect the pulmonary responses to particles.

In addition to the interdependence of effects of particles and gases in mixtures, particulate material itself exhibits effects on pulmonary function. Other than frank pathologic changes from chronic exposures to toxic materials such as asbestos and silica, and reactions of lung tissue to prolonged exposure or high concentrations of salts of such metallic compounds as Al and Cd, the less dramatic physiologic responses to dusts as part of a pollutant mixture may not be ignored. A few breaths of relatively innocuous dusts at low concentrations can produce a significant bronchoconstriction (124, 125). A frequent or steady exposure to small concentrations of particles must be taken into account along with any other etiologic factors in exposures to mixtures of atmospheric pollutants.

Selected variables in pulmonary response.—Attempts to assess pulmonary effects of air pollutants and other airborne toxicants are complicated by a number of variables. The enhanced susceptibility of asthmatics to particulate material and irritant gases has been established experimentally and continues to garner epidemiological support throughout the world (126). It is not unreasonable to predict that other disease states with less obvious and less direct reaction to air pollutants will also indicate individuals with heightened susceptibilities. At the other end of the spectrum the newborn is less prone to respond to agents that produce edema (127) and the young are less susceptible to oxidants, perhaps because of their greater surfactant reserve (111). Although variability with regard to sex has been established for mixtures of compounds, specific basic differences in response to individual agents are lacking. Therefore one must hold, tentatively, that effects of airborne insults on the lung are so complex that, at best, a trend only exists toward a higher susceptibility of males to major pulmonary and related cardiovascular diseases. Clear differences will require separation of many other contributory factors.

The role of antoxidants such as Vitamin E in protecting experimental animals from effects of air pollutants (71) suggests diet as an important variable. Other studies also imply a dietary dependence such as the increase of ozone effects on cells from starved animals and the requirements implied for the biosynthesis of surfactant. Cellular integrity of phagocytic elements may be directly or indirectly dependent on diet, and even ciliary clearance may be influenced by diet, as implied by the work of De Luca (128) who demonstrated a decrease in mucous secreting cells in Vitamin A deficiency. A factor that promises to be the most telling variable is the genetic or constitutional prediliction for diseases of the respiratory tract alluded to above.

One variable has received, deservedly, the most attention, viz, smoking. Aside from the mass of literature on the effects of smoking, particularly the contribution of smoking to emphysema, there are parallels that bear emphasis. Well over 200 compounds are present in cigarette smoke; in effect, smoking represents a special kind of concentrated exposure to an air pollutant atmosphere. The possible roles of many of these compounds in the etiology of diseases associated with smoking have been discussed during a symposium entitled "Toward a Less Harmful Cigarette" (NCI Monograph 28). Just as there is difficulty in identifying causative agents in air pollutants, so the same difficulty besets us in seeking the agent(s) responsible for effects of smoking. Nitrosamines (129), benzpyrene (130), radioactive substances (131) all are known initiators of carcinogenesis; oxides of nitrogen and carbon monoxide in cigarette smoke, free radicals (132), and other classes of chemicals with known effects (130) all fail a full test as a primary causative agent. Even a simple separation of "aerosol" and volatile constituents in terms of effects has not been possible according to the carefully reasoned presentation by Rylander (133). There is no question of the effects of smoking and there can be no doubt that a predominantly synergistic relation with air pollution exists.

Particulate Material

The series of diseases categorized under the broad heading of pneumoconioses has been well covered in the literature. A recent surge of interest has occurred in the alarming recognition of the high incidence of "asbestos" bodies and their relation to pulmonary disease in city dwellers. In a series of papers devoted to effects of fibrous dusts such as ceramic glass, talc, and asbestos, Gross has provided a method for separation of "ferruginous" bodies, which he discusses as possibly of origin distinct from asbestos fibers, and has compared the pulmonary response to a variety of fibers (134–138). The relation of asbestos exposure in the presence of other materials to the incidence of neoplasia in the population remains subject to further study despite the energetic efforts of workers in this area.

The metals, which comprise another prominent class of particulate material in the atmosphere, have a manifold significance. For example, the "balance" of metals is altered in aging and in neoplastic changes (139, 140) sug-

gesting a relationship of the metals to the disease process and to susceptibility to disease with age. Second, close, perhaps causative relations may be found for metals and diseases such as Cd and hypertension (141, 142). The essentiality of some metals appears well established; consequently the intake of other metallic compounds may be expected to alter function by changing the net constitution of metals available in the body.

Insoluble metallic compounds, particularly oxides, have effects on the lung simply related to their particulate nature, may produce pathologic responses in pulmonary tissue, or may be synergistic with other compounds in such effects as susceptibility to infection via effects on phagocytosis and direct effects on surfactant material (143).

The presence of oxides of metals in the atmosphere raises questions which can be illustrated by a brief consideration of Pb. Despite the years of research on Pb and its control, several points of controversy remain. Despite the fact that a major portion of Pb in humans is from dietary sources as emphasized by Kehoe (144), the respiratory intake is often cited as a problem area for the general population exposed to automotive exhaust. Haley (145) in describing the "supposed chronic lead inhalation" from inhaled lead as a "myth" cites the small dose to the respiratory tract from products of leaded gasoline and the fact that the marked increase in atmospheric lead has not altered the body burden in the human over many years. At the same time one might suggest that with the marked decrease in products that have contributed to the oral dose (e.g. lead pipes) one might have expected a decrease in body burden during the same period. The work of MacDonald on sequestration of Pb in bone (146) is cited as further support for the lack of hazard from Pb in the atmosphere. On the other hand, movement of Pb into bone as part of the steady state of Pb in the body may be invoked as a danger to the acceptance of blood levels as an index of Pb toxicity, a contention for which Kehoe has provided ample support (147) although it is not a unanimous view. Indices of metal toxicity (e.g. for Pb, delta amino levulinic acid in the urine), are useful but do not uniformly indicate chronic toxicity or ultimate mechanism of effect. Susceptible segments of the population such as those carrying G-6PD deficiency further complicate prediction.

Similar sets of questions can be raised for many metals and metallic salts of limited solubility which are found in the environment. One key question for particulate material entering the body by way of the respiratory tract is the accessibility of the material to the site of toxicity in the lung or at a nonrespiratory site. There is no a priori reason to expect particulate compounds in the lung to mimic the distribution sites and paths followed by an oral dose. Indeed one could conjure a variety of reasons for the distribution to be different. In dealing with particles in the atmosphere, it is absolutely essential to face the potential toxicity fully armed with fundamental information on both the parameters of deposition and the pathways and mechanisms of pulmonary clearance.

Deposition.—The deposition of particles in the respiratory tract is subject to many variables. Physical parameters obviously must include particle size as a major determinant of the site of deposition. Physical size is not as important however as the aerodynamic size of the particle, approximated by a combination of physical size and density. Other parameters include hygroscopicity of the material which determines the growth of size as the particle traverses the water-saturated atmosphere of the respiratory tract, shape of the particle, particle concentration which is directly related to coagulation, and the relation of the particle to other components in a mixed atmosphere. In most atmospheres, whether naturally occurring or generated, there is a distribution of sizes that usually approaches a "log-normal" pattern.

Biological variables fall roughly into two compartments—anatomical and physiological. The former includes a distinction between mouth and nose breathing, the shape, sizes, and branch angles of the respiratory tree, and the geometric change during respiration. Physiological parameters of greatest moment are the combination of inspiratory velocity, tidal volume, and frequency (e.g. related to degree of work), presence and duration of respiratory pauses, and the degree of response of the tracheobronchial tree to the material during inhalation.

Respiratory deposition of particles and influences on the processes have been subjected to many treatments often for different purposes. The influences of particle size on regional deposition in man have been examined by Lippmann & Albert (148) and a full treatment of the concept and mechanics of assessing the "respirable" fraction of dust population has been presented, in tandem, as a Guide for Respirable Mass Sampling by the Aerosol Technology Committee of the American Industrial Hygiene Association (149) and documentation by Lippmann (6). Instruments and techniques have been recommended (149). These and other recent papers emphasize that the assessment of hazard to the general public, or to any segment of the population must be based not on the total mass in the atmosphere but on that fraction that is of biological significance.

Both physical and physiological variables are well reviewed and interpreted by the ICRP Task Force on Lung Dynamics (13). Starting from experimental data, the Task Force has generated computer data for regional deposition of a wide spectrum of particle size distributions [Mass Median Aerodynamic Diameter (MMAD) from 0.01 to 100 μ with geometric standard deviations of 1.2 to 4.5] at tidal volumes of 750, 1450, and 2150 ml representing mild to heavy work. Within these wide variations of particle size the fractional deposition between nasopharyngeal, tracheobronchial, and alveolar ("pulmonary") regions of the lung falls within surprisingly narrow limits ("envelopes") for a given tidal volume and MMAD. Thus the MMAD is the only measure of particle size that provides a simple and reasonable basis for prediction of the probability of regional deposition applicable to the entire particle distribution.

The mean deposition over a wide particle range also changes relatively

little for either the whole respiratory tract or the nasopharyngeal and tracheobronchial areas as the tidal volume is changed from 750 to 2150 ml. Alveolar deposition however, at least by this model, is predicted to decrease markedly with decreased tidal volume below MMAD of 0.10 μ and increase with lowered tidal volume at MMAD greater than about 0.8 μ. These and other relationships, although subject to further modification, represent a major effort in producing a generally applicable lung model for deposition of particles.

In relation to site of action in the lung or, in relation to toxicity of a material, the regional position of deposited particles has several levels of significance. Obviously the site of deposition and the quantity of material deposited are at least partial determinants of the site and degree of response. For materials of differing solubility the particle size has a special relation. With a given total atmospheric concentration with a predominantly large particle size, deposition in the upper reaches of the respiratory tract will be of less potential toxicity than a more finely divided population which will be deposited to significant extent in the depths of the lung. Conversely with a readily soluble material the large particle size will yield a greater *total* deposition to surfaces from which absorption can occur and the systemic dose will be larger. Another particular point of significance, among many which could be cited, is the relation of the site of deposition to the clearance of particles from the respiratory tract.

Pulmonary clearance.—Particles deposited on ciliated epithelium are subject to clearance by the movement of the "mucous blanket" up the respiratory tree to the glottis, a process that is rapid (min.-hr.). An early rapid clearance is often referred to as phase I clearance (13, 14). It may, in fact, be divided into several sub-phases related to nasopharyngeal clearance, clearance of major subdivisions of the tracheobronchial tree, and clearance of material deposited in the finer bronchioles. This phenomenon is readily apparent qualitatively as cycles of appearance of particles in various size airways (150) and is suggested by quantitative measurements of clearance (151). It has been suggested that some participation by readily mobilizable macrophages is also a part of the first phase clearance (152); it is also contended that phagocytosis is not likely to be contributory to this phase (153). In the mammalian lung, phagocytosis is certainly not prerequisite to the rapid phase of clearance but it is probable that incidental phagocytosis occurs by cells that have already reached the ciliated epithelium prior to contact with particles deposited at those sites. In any event, the latter part of ciliary clearance overlaps subsequent phases of clearance (14).

As alveolar clearance proceeds without the immediate involvement of cilia, a central process is phagocytosis. The time course for removal has been stated to be highly variable (13). However, this contention is partially based on inclusion of all clearance subsequent to Phase I as a single second phase instead of recognizing multiple components of the clearance phase. If

the initial rates of clearance in a second phase are examined, the rates for a variety of comparable particle species are, in fact, not very widespread. The case has been presented for a cellular rate-determining role in clearance during this phase (9, 14, 76).

The rates of clearance are dependent on several factors. The cytodynamic capability of the source of phagocytic cells, the maintenance of functional integrity, the rate of production of cells, and their degree of mobility all may influence the extent of clearance with time. These factors are influenced in turn by the degree of cytotoxicity of the particle, the total amount of dust deposited, and the number of particles in relation to cells. The latter conclusion, although deduced from in vitro studies of alveolar and peritoneal macrophages, is reasonably applicable to the in vivo situation (93, 154).

The origin of the cells contributing to the phagocytic pool in the lung, the precise mechanics of the cell-particle interaction and the roles of the cells in pulmonary effects collectively must constitute a separate topic for review. A number of reviews provide emphasis for specific aspects of alveolar clearance (7, 9, 13, 14, 76, 77).

Research Horizons

A workshop in Pulmonary Toxicology[2] conducted under the sponsorship of the Division of Research Grants of the National Institutes of Health resulted in a number of recommendations for areas of support (155).

The atmosphere.—It was concluded that the more common methods of producing experimental aerosols are unsatisfactory, among other reasons, because they produce a wide spectrum of sizes and there is a lack of reproducibility of particle populations from one experiment to another. Methods in use are compromises. Despite some current attempts to arrive at better methods of aerosol generation, expansion of these efforts was recommended. In the broad context of the characterization or identification of airborne materials, it was observed that in field studies, particle size is not always given proper consideration. When it is measured, the data are not usually optimally related to toxicological interpretation. Conversely, laboratory studies frequently entail characteristics not readily applicable to real atmospheric circumstances. Manual and visual methods used in the laboratory often incorporate some "error" related to the collection and measuring technique itself and the methods are tedious and time-consuming. Although current methods of automation are often easier and faster, they incorporate a number of additional errors which are not always readily apparent to those who are using the instruments.

An obvious conclusion was that the parameter of the particle population

[2] Participants: M. O. Amdur and L. J. Casarett, Co-chairmen, J. A. Clements, J. Doull, M. Kuschner, P. T. Macklem, R. S. McCutcheon, T. T. Mercer, and E. D. Palmes.

should be used that best reflects the basic toxicologic questions posed. When the question relates to an experimental evaluation of circumstances known to occur in the field, the measure in the laboratory should properly relate to the particle parameter inherent in the field sampling. As a minimal requirement for both laboratory and field studies it was recommended that one must have a measure of the "respirable fraction" of the particle population at least as a two-stage partition. Recommendations for instruments and techniques have recently appeared (149).

Deposition and physiologic response.—Despite the availability of a great deal of information on deposition in the literature, much of it is compartmentalized. As there is too little crossover among those engaged in separate areas of interest, one finds questions raised in one area of research, the answers to which can often be found elsewhere in the literature.

The use of lung models to predict deposition patterns has some value when the model has been carefully constructed (13). This value, however, is limited to the derivation of an approximation on which to base an experimental design. The actual pattern of pulmonary deposition must be obtained for any particular material and is subject to the influences of the means of generation and methods of measurement.

The use of homogeneous particle populations has some advantages in relatively sophisticated studies of respiratory deposition, among which are the following: (*a*) such a population permits a careful study of the deposition characteristics with a known size; (*b*) clearance kinetics from chemical or radioactive analysis allow translation of the data into particle number; and (*c*) more clear-cut interpretations are possible without the complications of a polydisperse aerosol provided one can reproduce a particular size from one experiment to another.

The contention by proponents of the homogeneous aerosol that one can reconstruct, from a summation of individual studies of monodisperse populations, what would occur with a polydisperse system constituted of similar mixed sizes, cannot be substantiated adequately. Furthermore, the homogeneous aerosol does not represent the field situation closely enought to validate application of toxicological data. It was concluded that monodisperse aerosols are of advantage to study fundamental deposition processes and for specific experimental toxicological applications, while the more realistic polydisperse system if it is sufficiently reproducible is preferable for study of inhalation toxicity.

A central theme of physiologic response concerned the degree of reliability of animal tests in predicting human response, and corollary questions. There are unquestionably differences among species and variability within species in most or all of the physiologic measures of response customarily used. Further, a measure of response such as a change in maximal expiratory flow rate (MEFR) often does not parallel changes in other measures such as pulmonary resistance. It was suggested that various mea-

sures of physiological change are associated with events at different parts of the respiratory tree.

Two particular facets were singled out. Partly because of the difficulty of measuring changes there is no convincing evidence that constriction of peripheral airways in humans results in changes in the elastic properties of human lung as occurs in animal species. Studies of resistance and examination of the question of changes of elastic recoil in human lung, if any, would be of value. A second facet is that of collateral ventilation, for which there are few usable data in humans comparable to those in animals. It was further suggested that the degree of collateral ventilation in man should be viewed with aging and in disease.

Two corollary recommendations were made for additional research. One of these was the regional response of lung particularly with varying deposition patterns; the other was the separation of multiple actions of mixed atmospheres.

Pulmonary clearance.—Although much work has been carried out measuring the retention of materials in the lung, even this minimal estimate of the true dose to the respiratory tract is lacking in many studies. Furthermore, less effort is devoted to fundamental aspects of the mechanisms of clearance and to the cellular and macromolecular interactions of noxious agents with components of pulmonary tissue than is warranted by the complexity and importance of alveolar responses. Among the needs singled out and recommended for support were studies of means for measuring pulmonary clearance in humans and for derivation of data for use in arriving at pulmonary concentration-time values for estimates of dose; continued attack on alveolar regions of the lung at refined biochemical levels; studies of the role of cells of the alveoli in clearance, response to noxious substances, and in the etiology of disease processes and the interrelations of the cellular and noncellular components of the pulmonary parenchyma.

Pathologic effects.—Many observations were offered in this area, of which only a few may be mentioned. Simple descriptive pathology is toxicologically inadequate. In lethality experiments, examination of the acute anatomic changes should be accompanied by inquiry into the mechanism of death. There should also be more consistent examination of the persistence of the acute changes in the survivors. Many studies do not distinguish delayed or chronic events clearly as to whether, for example, they represent increased incidence of a normally lethal lesion, an earlier onset of a frequently or normally lethal lesion or a specific lesion not normally found. Further, a degree of specificity of effect may be attained by more assiduous identification of the site, character, and pattern of changes than those customarily examined, e.g., qualitative and quantitative changes of specific lung tissue components.

Epidemiological and laboratory toxicology.—A basic problem is that of

securing predictive information on adverse actions of inhaled materials on the human respiratory tract. Present laboratory procedures in inhalation toxicology and epidemiological methods are directed toward establishment of a dose-response relationship. A major difference in the two approaches lies in the precision with which dose and response can be measured. In the laboratory, the dose can be closely estimated and is predictive for acute effects but may not be applicable in chronic exposures, among other reasons, because the responses that can be measured are not necessarily the ones of greatest concern to the chronically exposed human population.

Among the recommendations made, two are of pertinence. One of these was to recognize the necessity for vertical studies extending beyond the lifetime of the investigator and for devising means to maintain such programs. As this represents an expensive, tedious approach requiring commitments not often attractive to modern investigators and institutions, alternate suggestions were made. One of these was to support expansion of the search for provocative tests which could provide early indices of response. Such a measure of susceptibility combined with a more intense search for biochemical indices of genetic susceptibility may serve to obviate, partially, the time-consuming lifetime studies.

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