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IN

TOXICOLOGY

I

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CHAPTER 3

INHALATION TESTS

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PURPOSES OF INHALATION TESTS

Inhalation tests are difficult to conduct and interpret. It is therefore important that those who embark on them should have a clear idea of the questions to which they seek answers. Valuable information may often be more easily obtained from less specialized techniques. The examination of a material specifically for respiratory hazard requires a different approach from that needed in the establishment of general safety of the same material.

Most inhalation studies are undertaken for one of the following reasons:

Detection of local toxicity to the respiratory tract, including the nose, nasal sinuses, nasopharynx, larynx, trachea, bronchi and lungs.

Detection of general toxicological hazard.

Establishment of safety-in-use.

Enquiry into the factors and the study of mechanisms involved in disease of respiratory tract, especially lung cancer.

Basic studies of lung structure and function.

As for any laboratory tests, each should be tailored to the particular material and problem. For this purpose all available and relevant information with regard to the test material, including information from other toxicological studies, should be taken into account whenever an experiment is designed. It is especially important that the physical and chemical specifications of the test material be known and, where necessary, identical with those of that to which man is exposed.

MODELS FOR THE DETECTION OF INHALATION HAZARD

Inanimate and *in vitro* Systems

The study of the aerodynamics of particles of various shapes, sizes and specific gravities is well advanced (Hatch and Gross, 1964; Green and Lane, 1964; Davies, 1949, 1952, 1961, 1966; Wilson and La Mer, 1948). This knowledge, and a detailed knowledge of the

TABLE 1.

Examples of Factors which Influence the Deposition and Retention of Particles in the Respiratory Tree

Physical and Chemical Characteristics of Particles

(*a*) Size, density, viscosity and shape of particles.
(*b*) Electrical charge (Whitby and Liu, 1966), coalescence, volatility and hygroscopticity (see Green and Lane for review, 1964).
(*c*) Inertial forces (i.e. particles tend to continue in same direction and at same speed despite changes in direction and diameter of airways), settlement by gravity, Brownian movement.
(*N.B.* Simple contact between particles and walls is not an important factor.)
(*d*) Solubility in body fluids.

Anatomical Factors

(*a*) Variations between individuals in body size, shape of nasal cavity and thorax and in anatomy of respiratory tree.
(*b*) Variation in distance (7–25 cm.) and numbers of generations of airway (15–25) between trachea and alveolus in different bronchopulmonary segments.

Physiological Factors

(*a*) Nose or mouth breathing, speaking, singing, etc.
(*b*) Changes in rate of airflow and reversals of air flow.
(*c*) Greater expansion and contraction of apices than bases, especially during quiet respiration.
(*d*) Changes in diameter of airways during respiratory cycle.
(*e*) Degree of mixing of old with new air.
(*f*) Effect of cardiac pulsation. According to West (1961) this causes sudden accelerations and decelerations of air and its movement from one part of the lung to another.
(*g*) Ventilation rate, which is itself dependent on severity of exercise, age, bodyweight, training for particular task, etc. Ventilation rate not only affects the volume of air inspired and the rates of airflow in the numerous airways, but also the proportion of the tidal flow which acts as ' dead space ', the degree of mixing of new with old air, whether air is inspired through the nose or mouth, and the proportion of the lung in full use, etc.
(*h*) Breath-holding time (Boyland, McDonald and Rumens, 1946).
(*i*) Thyroid function (Fairchild, Murphy and Stockinger, 1959).

Pathological Factors

(*a*) Nasal blockage.
(*b*) Excessive production of mucus.
(*c*) Decreased number or efficiency of cilia.
(*d*) Reduction in airway size.
(*e*) Raised ventilation rate because of anaemia, emphysema, etc.
(*f*) Impairment of blood flow through the lungs and reduced capacity for gaseous exchange through alveolar walls, as in emphysema, pulmonary fibrosis, etc.
(*g*) Blockage of lymphatics (e.g. by previously deposited particles).

anatomy of the respiratory tree, should allow one to predict where the inhaled particles will be deposited. But accurate prediction is not as simple as this on account of the multiplicity of factors involved. Some of these are listed in Table 1. Models of the human respiratory tree, such as that of Ermala and Holsti (1955) and West (1961), enable the prediction patterns of particle deposition to be tested experimentally. In general, the anatomical model provides reasonably accurate information for the large airways, but is of little use for the smaller ones particularly because they change in diameter during the respiratory cycle (*see also* Watson, 1953).

Even less predictable is the absorption of deposited material at, and removal from, the sites where it is initially deposited. *In vitro* systems are available for the study of the effects of agents on ciliary motility. Battista (1962) pointed out that drying of a preparation, such as the frog's tongue or rabbit's tracheal mucosa, may result in ciliary stasis. Recovery from this paralysis takes place after variable intervals *in vivo*, so that the consequential delay in the removal of deposited particles may vary widely. Bernfeld, Nixon and Homburger (1964) have emphasized the problem of drawing meaningful conclusions from *in vitro* tests of ciliary activity.

Light on carcinogenicity may be reflected from studies of cells of the respiratory tract maintained in tissue or organ culture (Lasnitzki, 1956, 1958; Laws and Flaks, 1966). These systems cannot be regarded as a substitute for general toxicological or carcinogenicity tests on living animals. An inherent limitation of this method is the absence from the system of metabolizing tissues, particularly the liver, which may convert inactive carcinogenic precursors to active proximal carcinogens.

Animate Systems, including Human Studies

Unquestionably the best model for toxicological studies relevant to man is man himself. In practice, it is usually unjustifiable to subject individuals to unknown hazards, although full use should be made of information which may have become available before dangerous potential of the material was recognized, or subsequently as a result of accidental contact. Unfortunately, exact details of exposure are often lacking in practice, particularly where the manifestations of toxicity are delayed. In such instances it is common for individuals to have been exposed to a variety of potential hazards by a variety of routes. Individuals vary widely in respiratory behaviour and efficiency, even after standardization for age, sex, height and weight.

Deliberate studies on man, such as that by Morgan (1965) on ^{132}I-labelled methyl iodide, have been very informative, particularly in relation to testing the predictions of patterns of particle deposition based on aerodynamic calculations and to studies on the absorption and removal of inhaled materials from the respiratory tree (Davies, 1961). However, such methods are usually of limited value for detecting the hazard of newly introduced inhalable materials.

Necropsy studies on man may reveal the site of retention within the lungs of identifiable inhaled material, e.g. radioactive materials of long half-life, metals, asbestos, and of the distribution of pathological changes arising from inhalation. For several reasons, however, this information is usually of limited value: the amount retained may be only a small and incalculable fraction of the total to which the individual was exposed and the points of accumulation of retained particles (e.g. the hilar lymph nodes) are insufficiently indicative of the site of initial deposition.

So, in general, for the investigation of inhalation hazards, there is no practical alternative to tests under controlled conditions on laboratory animals. The choice of species then depends on the objectives of the experiment. In some cases the similarity of the size and structure of the respiratory tree of the model to those of man may be of paramount importance, whereas for long-term studies the need to use a species of small body size may be the determining factor.

Dosimetry problems may be circumvented, particularly in short-term studies, by anaesthetization of the animals during inhalation exposure (Ulmer, Reif and Biebricher, 1961). However, anaesthetics and narcotics may profoundly alter both the deposition pattern of inhaled particles (Fleck and Edery, 1961) and the metabolism of deposited material.

Features of Animal Species Commonly Used for Inhalation Exposure Studies

Anatomy of Respiratory Tree and Chronic Inflammation

Roe and Walters (1965) compared the respiratory systems of various laboratory animals with that of man in search of a model for the study of the aetiology of human lung cancer. All the species small enough for large-scale long-term studies were found to differ markedly from man. First, they differed in the factors which affect particle deposition, e.g. anatomy of nasal cavity, airway size, degree of tortuosity of airway system and rates of air flow. Second, they differed in their mucus secretion; thus mice, hamsters and rabbits possess virtually no tracheal or bronchial mucus-secreting glands

and rely entirely on surface goblet cells for the provision of mucus. All strains of rat are similarly deficient in bronchial glands, though some have a moderately good mucus gland development in the trachea. Roe (1966) pointed out that the distal end of the main bronchus in the mouse, which has neither cartilage nor mucus glands, is comparable with the sixteenth generation airway in man. Cats have abundant glands in the bronchial walls but these, unlike the surface goblet cells, stain poorly with PAS and produce a thin serous type of secretion. The abundant production of the latter in response to a variety of respiratory irritants renders the species generally unsuitable for respiratory studies. The guinea-pig has mucus-secreting glands, but its predisposition to asthmatic-type spasm on exposure to irritant materials is a drawback for most experimental purposes.

Different breeds of dogs and species of monkeys more closely resemble man both in gross anatomy and in spectrum of response, though they vary in the extent to which bronchial mucus glands are developed. These species should certainly be considered for small-scale, short-term experiments, but their size virtually precludes their use for large-scale or long-term studies. The same limitation applies, *a fortiori*, to the use of the horse (Tyler, McLaughlin and Canada, 1967).

An obvious requirement for any model is that it should be capable of mimicking the human disease state which is the primary object of study. Despite the anatomical differences referred to above—in particular the lack of mucus glands in many rodent species—the rat may act as a suitable model for the study of factors which predispose to chronic bronchitis in man (Reid, 1963). Similarly, according to Freeman and Haydon (1963), the rat is capable of developing ' dry emphysema '. Bronchiectasis is an all-too-common spontaneous condition of rats (*vide infra*) and although the histopathological appearances both of bronchiectasis and of other chronic inflammatory and fibrotic states in the rat have features different from those of man, the similarity is probably adequate for most purposes.

According to Tyler, McLaughlin and Canada (1967) the suitability of the model should be examined at the submicroscopic as well as at the macroscopic and microscopic levels.

Neoplasia of the Respiratory Tree in Mouse and Man

The common types of pulmonary neoplasm in various species show quite marked differences between rodents and man. Virtually all spontaneous primary tumours of mouse lung are adenomas and adenocarcinomas, although whether they arise from

alveolar or bronchiolar epithelium, or from both, is disputed (Grady and Stewart, 1940; Mostofi and Larsen, 1951; Orr, 1947). Andervont (1937) and Andervont and Shimkin (1940) induced squamous carcinomas in mice by threading strings saturated with carcinogenic polycyclic hydrocarbons through the lungs, and Gates and Warren (1961) achieved the same result by exposing mice to gamma radiation from a ^{60}Co point source introduced into the lung or pleural cavity. Straub (1940) and Rickard and Francis (1938) drew attention to the marked proliferative response of mouse lung to infection with influenza virus. Kotin and Wiseley (1963) exposed C57 black mice to four different strains of influenza virus and then to an atmosphere of ozonized petrol. Whether the laminated epithelial changes seen in these experiments were neoplastic or metaplastic was difficult to determine, but the authors considered that some of the lesions were squamous carcinomas. Harris and Negroni (1966) undertook a similar experiment on C57 black mice which they exposed to influenza viruses followed by tobacco smoke. A low incidence of lung tumours resulted, mostly adenocarcinomas, but two had squamous elements. On transplantation the squamous elements disappeared and only the adenomatous pattern was reproduced. Horton, Tye and Stemmer (1963) claimed to have produced epidermoid carcinomas by causing mice to inhale formaldehyde vapour plus an aerosol of coal tar. Gates and Warren (1961), who induced squamous cancers in two mice by exposing them to an alpha-particle-emitting plutonium compound, pointed out that the production of such tumours in mouse lung seems to require prolonged and intimate contact between carcinogen and lung tissue.

Strains of mice differ widely in proneness to develop adenomatous lung tumours, either spontaneously or in response to carcinogenic stimuli (Heston, 1948). To induce epidermoid tumours it is probably necessary to use a strain with a low susceptibility to the adenomatous type of tumour; otherwise the development of multiple tumours of the latter type is likely to obscure the picture. This was the reason for the choice of the C57 black strain by Kotin and Wiseley (1963) and Harris and Negroni (1966).

In summary, it is clearly difficult to induce squamous carcinomas in the lungs of mice, and the induction in the mouse of what is probably the most interesting type of lung cancer in man, the oat-cell carcinoma, has not been described. Roe and Walters (1965) argued that the lack of similarity between the histological types of pulmonary neoplasm in the mouse do not make it unsuitable as a model. On the contrary, the high susceptibility of mouse lung to the induction of pulmonary adenomas and adenocarcinomas may be regarded as

making it the tissue of choice for the detection of carcinogenic activity.

Neoplasia in Other Species of Laboratory Animal

In general the rat is not so prone to develop pulmonary tumours as the mouse (Saxton and his colleagues, 1948; Horn and Stewart, 1952). However, the tumours that do arise are mostly of the same adenomatous variety as in the mouse. Roe (1966) has reviewed briefly the ways in which lung tumours of both adenomatous and squamous varieties have been successfully induced in rats.

According to Saffiotti and his colleagues (1964) spontaneous lung tumours are uncommon in the Syrian Golden hamster. Della Porta, Kolb and Shubik (1958) induced both adenocarcinomas and squamous carcinomas by the intratracheal instillation of 7,12-dimethylbenz(a)anthracene in collodial suspension. This and the results of other experiments with benzo(a)pyrene and diethylnitrosamine (Herrold and Dunham, 1962, 1963) rather suggest that the trachea and main bronchi of the hamster are especially susceptible to the induction of squamous neoplasms in response to chemical carcinogens.

Shimkin (1955), in a review of the literature concerning pulmonary tumours in rabbits, could find records of only four cases—all adenocarcinomas. Franks and Chesterman (1962) recorded four cases of papillary adenomas, but no other neoplasms, in the lungs of 255 guinea pigs.

Nielsen and Horava (1960) found reports of 103 primary lung tumours in dogs. Of these, 63 were adenocarcinomas. In their own necropsy material from over 9000 dogs, they encountered 16 pulmonary neoplasms of which 12 were bronchiolar adenocarcinomas and one a bronchiolar adenoma. Squamous carcinomas also occur in dogs (Monlux, 1952) and in recent years their induction by chemical carcinogens has been reported (Rigdon and Consen, 1963; Beattie and his colleagues, 1961).

There is at present little information on the occurrence or incidence of pulmonary neoplasms in monkeys. Because of the lack of such background information and for other reasons (e.g. lack of precise knowledge of age, genetic constitution and pathogen burden), it would for most purposes be unwise to choose monkeys caught in the wild for long-term inhalation studies. Only where laboratory-bred monkeys are available in adequate numbers is their use for inhalation experiments feasible. As basic information on such animals accumulates, they may become the species of choice for some types of inhalation studies.

Exposure to Gases

Except in the case of gases which react with oxygen (e.g. nitric oxide), or with water (sulphur dioxide, nitrogen dioxide, ammonia), the examination of the effects of the inhalation of gases is comparatively easy. The inflow of the gas from a compressed source is easy to meter and the monitoring of the concentration within the exposure chamber generally presents no problem. Strafford, Strouts and Stubbings (1956) reviewed methods for the determination of various noxious gases and vapours in air and the effects, on certain animal species, of exposure to gases such as carbon monoxide, oxides of nitrogen, sulphur dioxide, ozone, are discussed in the Surgeon General's Report to the U.S. Congress (1962). Most of the work referred to in this report is, however, concerned with the effects of relatively short-term exposure and there is a need for more long-term studies, such as that of Freeman and Haydon (1963) who produced dry emphysema in rats by exposing them over a long period to nitrogen dioxide.

Where the test gas interacts with water, control of exposure becomes more complicated. As a rule the saturation point for the reaction product is low, so that it condenses to form a mist. If this happens, the problem of control of exposure condition entails measurement of droplet size and measurement of cloud density as for exposure to aerosols.

Intratracheal Instillation and Direct Injection into the Lung

Blacklock (1961) and Blacklock and Burgan (1962) induced lung tumours in rats by the injection of tobacco smoke condensate directly into the pulmonary tissue of rats. Similar experiments in rabbits and guinea pigs led to epithelial metaplasia and *in situ* carcinomas, but to no frankly malignant tumours. Such a technique is far from elegant and *a priori* one would doubt its relevance to inhalation exposure. Kuschner and his colleagues (1957) induced cancer by attaching pellets impregnated with carcinogens by hooks on to the walls of rat bronchi.

Intratracheal instillation, either via the larynx (Della Porta and his colleagues, 1958) or by injection through the cricothyroid cartilage (Rigdon, 1960) is possibly closer to inhalation exposure than intrapulmonary injection, but has many limitations. The extent of exposure of different parts of the lung is uneven and the distribution of injected material does not necessarily bear any resemblance to that of inhaled materials.

Rockey and his colleagues (1958) described a technique of tracheal fenestration in dogs. Following this operation materials

could be introduced into selected areas of the bronchial tree through a tracheocutaneous fistula. Beattie and his colleagues (1961) induced invasive carcinomas in dogs by the repeated intratracheal instillation of a carcinogen after an operation to reverse the direction of ciliary flow in a portion of the trachea. However, the role of the latter operation is doubtful, since Staub and his colleagues (1965) achieved similar results using the same carcinogen but without this surgical manoeuvre.

Theoretically, gases could be introduced into the lungs in this way, but there are no reports of the method having been used in mammals, probably because it is unnecessarily complicated by comparison with simple inhalation exposure. Peacock (1955) pointed out that gases injected into the air sac of birds, such as the domestic fowl, are unavoidably inhaled by the bird.

Design of Exposure Chambers and Control of Exposure Conditions in Inhalation Tests

The basic requirements for the conduct of inhalation exposure studies as listed by Hinners, Burkart and Cöntner (1966) are set out in Table 2. The actual requirements vary according to the purpose of the tests and each experiment presents its own special problems. The successful conduct of an experiment entails close co-operation between workers in several disciplines, particularly between physicists, engineers, experimental pathologists, respiratory physiologists and microbiologists.

Inhalation exposure studies are usually best undertaken in specially constructed laboratories. These should provide abundant floor area with access to all sides of each chamber. The ceiling should be high and constructed so that pipes and apparatus may, if necessary, be suspended from it. The area should be well illuminated, but should receive no direct sunlight.*

The size of the chambers varies with the experimental need. For the even dispersal of particle clouds a cylindrical chamber would theoretically be superior to a chamber with a square cross-section. However, the latter is easier to make and is more convenient to work with. Also, except in the four corners, from which animals can easily be excluded, there is negligible variation in cloud density.

At the Sterling Forest Laboratories chambers varying in capacity from 10 litres to 1·3 cubic metres are in use. Those for long-term

* These conditions are well met in the department of Dr. Sidney Laskin at the A. J. Lanza Research Laboratories of the Institute of Environmental Medicine (New York University) in Sterling Forest, Tuxedo, N.Y. The present author is indebted to Dr. Laskin for providing the details given in this section and for permission to reproduce *Figure 1.*

studies are constructed of stainless steel and glass and have a smooth interior which renders them easy to clean. All animals within the chambers are housed in suspended wire mesh cages through which aerosol clouds may freely percolate. By this means, also, the animals

TABLE 2

Inhalation Exposure Apparatus: Basic Requirements

Exposure Chamber

(*a*) Adequate size for exposure of sufficient animals for statistical evaluation.
(*b*) Even distribution of test gases, aerosols or particle clouds.
(*c*) Temperature control.
(*d*) Humidity control.
(*e*) Easily cleaned and decontaminated.
(*f*) No hazard to personnel.
(*g*) Identical chambers available for untreated or vehicle-only treated controls.

Test Material

(*a*) In the case of gases—reliable control of concentration in chamber.
(*b*) In the case of aerosols and particle clouds—reliable monodisperse system as well as control of concentration. Vehicles, if used, should be without effect on respiratory system.

Monitoring of Exposure

Apparatus should be available for monitoring the concentration of gases, particle cloud density, and distribution of particle size continuously during exposure.

Animals

(*a*) Adequate supply of genetically homogeneous animals which are free from spontaneous lung disease.
(*b*) Facilities for microbiological control of animals.
(*c*) Facilities for lung function studies.
(*d*) Facilities for full pathological evaluation of effects of exposure.

Safety

(*a*) Where hazardous agents are under test, the absence of leaks in the chambers or connecting pipe systems should be ascertained repeatedly, preferably continuously, by a suitably designed monitoring system.
(*b*) The atmospheric pressure within the inhalation exposure system should be slightly below the ambient atmospheric pressure.
(*c*) Suitable traps and filters should guard both ends of all pipe-flow systems.

are prevented from reaching the corners of the chamber. Access to chambers is through a door which constitutes the entire front of the chamber. Aerosols are fed into chambers through a wide diameter pipe at the top of an inverted pyramidal funnel made of stainless steel (*see Figure 1*). Their withdrawal from the bottom of the chamber is effected by a similar construction. As a safety precaution, the

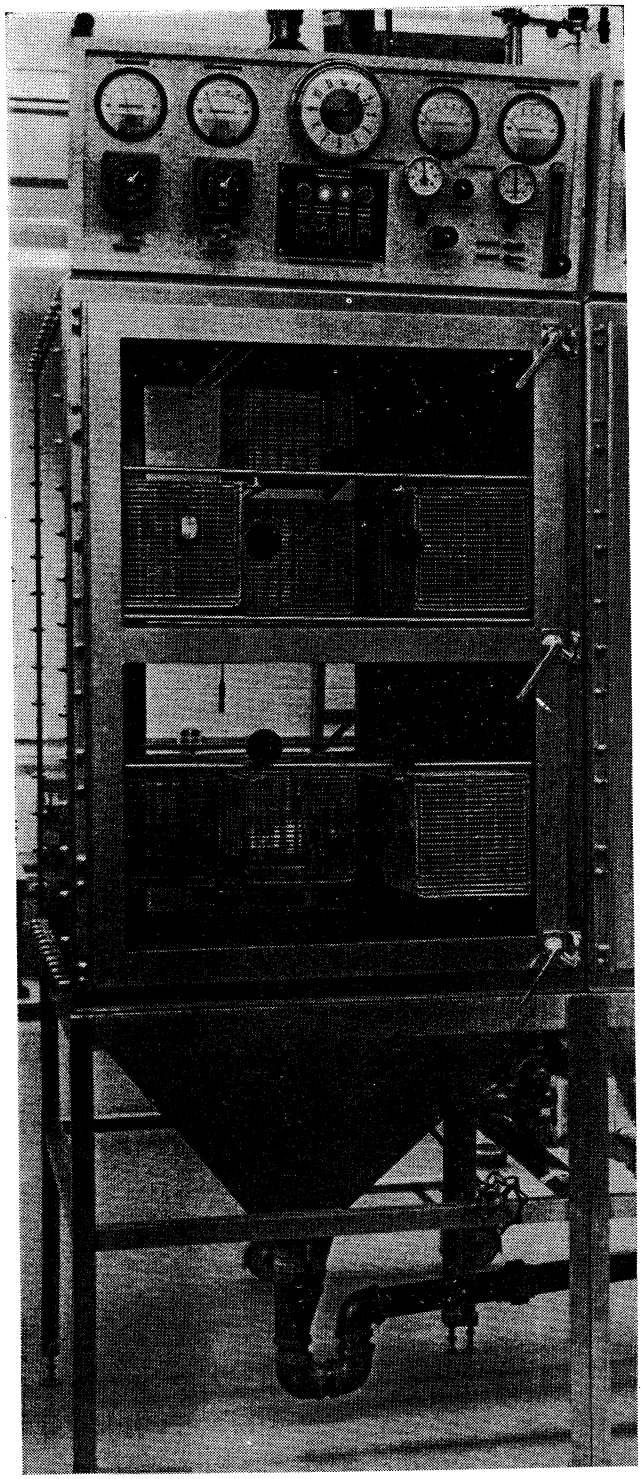

Figure 1. Inhalation chamber installed at the A. J. Lanza Research Laboratories (*see footnote on page 47.*)

chambers are kept, during operation, at a slight negative pressure (0·1–0·5 inches of water) relative to the atmosphere. The air flow from each chamber is measured by an orifice meter connected to a differential Magnahelic pressure gauge. Control of air flow and static pressure is maintained by means of manually operated gate valves. Intake air is temperature-conditioned and precleansed by passage through absolute filters. Air flow can be varied from 2 to 10 litres per minute for small units and from 2 to 20 cu. ft./min. for larger units. The rate of air flow and the temperature of the entering air can be balanced with the requirements of feed rate of the test material and the heat generated by the animals exposed. Total animal volumes are maintained at less than 5 per cent of chamber volumes to meet thermal balance requirements. Since the amount of air required for heat removal is usually far in excess of the volume needed for oxygen-carbon dioxide balance, this constitutes the practical lower design limit for air flow. The importance of the latter balance, however, is stressed by Glauser (1966) who found that hypoxia and hypercapnia gave rise to pathological changes in the lung parenchyma. A 5 per cent loading of animals efficiently fills the entire main body of the chamber with cages. Additionally, uniformity of exposure in terms of distribution and concentration of the test material can be well maintained at this level of loading.

The test material is introduced into a short ' T ' section at the top of each chamber designed to provide uniformity of mixing with diluent intake air. Exhaust air is drawn centrally from the bottom of each chamber passing through several stages of cleansing before entering the exhaust discharge system. The centralized exhaust discharge system at the Sterling Forest Laboratories is designed to maintain capacities of 1000–2000 cu. ft./min. under conditions of a fixed static pressure of 6–11 in. of water. Static pressure is maintained by means of a by-pass shunt system returning air to the duct from the discharge side of the blower and balanced by means of a weighted damper. Despite prior cleansing at each of the chambers, additional safety has been built into the exhaust system with the provision of large banks of disposable absolute filters. Standby filters and an accessory blower are also built into the system with electrical and pressure interlocks to provide automatic takeover in case of failure.

For some purposes it is not appropriate to house the entire animal body within a chamber. The skin of the mouse, for instance, is exceptionally permeable and intoxication may readily take place by absorption of noxious chemicals through it. Thus a mouse can be killed in less than a minute by the percutaneous absorption of

phenol. The deposition of radioactive particles on the skin of animal could also prevent the assessment of the effects of the inhaled particles. In such circumstances it is necessary to use an inhalation technique in which only the noses of animals are exposed to the test material. Various types of apparatus have been devised for this purpose (e.g. Lupu and Velican, 1957; Dontenwill, Reckzeh and Stadler, 1966; Harris and Negroni, 1966). In addition, various special techniques have been described which ensure that exposure is limited to the lungs (e.g. Peacock, 1955; Rockey, 1966).

Methods of Generating Aerosols and Particle Clouds

For detailed descriptions of the methods of production of aerosols and particle clouds the reader is referred to specialist books such as that of Green and Lane (1964). Basically, particle clouds are produced either by condensation from vapours, or by the dispersion into fine particles of substances in bulk or in a state of coarse subdivision. Condensation may be aided by the provision of nuclei and these may be insoluble or soluble, ionic or non-ionic.

Aerosols of many inorganic and organic substances may be prepared by dispersing the heated material from an open vessel in a stream of air. Such aerosols tend to contain particles of widely differing size, although the size range may be narrowed if efforts are made to control the conditions (Whytlaw-Gray and Patterson, 1932). Green and Lane (1964) give details of methods of preparation of monodisperse aerosols by slow condensation upon nuclei and by the mixing of vapour-laden gas streams at different temperatures. Ultrafine aerosols may sometimes be produced by the reaction of two chemicals both in the vapour phase, e.g. H_2SO_4 from SO_3 and water vapour or NH_4Cl from NH_3 and HCl. The rates of promotion of mist or smoke under such circumstances depend on the extent of the supersaturation of the product. If the product is hydroscopic the mist droplets may grow after their formation until an equilibrium between them and the water vapour pressure is reached. Other methods of aerosol production, based essentially on condensation, include generation by combustion, by electric arc and by photolysis.

Liquid atomization is an example of aerosol generation by dispersal. Three main types of device for liquid atomization are in common use: aerodynamical atomization in which a high velocity stream of compressed air or other gas is used to break up liquid emerging from a nozzle; centrifugation as, for example, by dropping a liquid on to a rotating disc; hydrodynamical atomization in which the liquid itself is forced through a nozzle and breaks up into droplets. Other methods include atomization by the use of electrostatic force

and acoustic atomization which utilizes high intensity sonic or ultrasonic vibrations. Of the aerodynamical kind of atomizers, the Colliston atomizer (Colliston, 1935) deserves special mention because of its widespread use. In common with similar types of apparatus, it incorporates a baffle to hold back coarser droplets and, when loaded with involatile liquids, almost all the droplets which emerge from it are smaller than 10 μ diameter, but the aerosol produced is polydisperse. Indeed, all these methods of atomization except the spinning-disc type give widely heterogeneous mists. The spinning disc, however, gives a remarkably uniform droplet size provided that the rate of feed of the liquid on to the centre of the disc is slow. Under such conditions the size of the droplet varies with the speed of rotation of the disc. Walton and Prewett (1949) used a self-balancing top capable of a very high speed for the purpose of producing very fine mists and May (1949) suggested an important improvement to this apparatus whereby unwanted satellite drops could be removed without the need for external suction.

Dust clouds may be produced in two basically different ways: by the dispersion of finely divided particles and by the disruption of solid matter. The earlier types of apparatus based on the first method consisted essentially of a system in which agitated dusts were carried away in a stream of air, but the clouds they produced were not very constant. Druett and Sowerby (1946) designed an apparatus in which the consistency was improved by the use of a tumble mill operated by a photoelectric cell to agitate the dust. The mill was switched on and off automatically depending on the optical density of the cloud in the chamber. Schrenk (1939) developed a system in which a test dust was steadily pushed up a narrow tube into a stream of air, but this had the disadvantage that aggregates of dust tended to emerge from the tube. Wright (1950) then arranged for the material emerging from the tube to be scraped from the top of the column in the tube by a rotating knife edge. This device can give relatively constant dust clouds provided that the input feeds into a relatively large chamber. The main source of inconsistency stems from uneven packing of the dust in the feed-tube.

Perkins and his colleagues (1952) developed an apparatus in which dust in compact form was eroded by a high velocity air stream. Provided that compaction is regular, this machine can produce a cloud of uniform particle size.

The significance of the shape of small particles in relation to their aerodynamic properties is discussed by Davies (1964).

The two essential requirements for radioactive aerosol studios are that the aerosol is chemically non-toxic and that the radioactive

tag is firmly bound to the particle. The special problems of the generation of such aerosols are briefly reviewed by Albert and his colleagues (1964, 1967) who also discuss potentially useful radioactive tracers.

Monitoring Exposure Conditions: the Measurement of Particle Size and Cloud Density

Even where the method chosen for the generation of an aerosol is reliable and controlled, it is essential to monitor both particle size and cloud density continuously during inhalation exposure experiments. An up-to-date review of the types of device available for this purpose is provided by Hatch and Gross (1964). A problem with most sampling devices is that the act of collection of the sample is liable to affect particle size and distribution (Drinker and Hatch, 1954). This is particularly true of cyclone collectors and impinging devices in which strong dynamic forces are used to bring about particle deposition within a limited space. In this connexion, Davies, Aylward and Leacey (1951) pointed out that it is neither necessary nor desirable to employ a jet of very high velocity in the course of air sampling. Only in the case of purely optical sampling devices (*vide infra*) is it possible completely to avoid some interference with the aerosol or particle cloud itself.

Most sampling devices, apart from those working optically, are based on a primary separation into particles likely to be deposited in the upper and lower parts of the respiratory tract. Those particles which are likely to penetrate into the lower respiratory tract form the ' respirable fraction '. Separation is based on size, and particles larger than 7–10 μ diameter are assumed to be non-respirable.

A convenient method for the removal of coarse particles is gravity settlement. As a polydisperse aerosol is passed in laminar flow along a horizontal channel particles settle early or late, depending on their size. An instrument based on this principle is the ' elutriator ', discussed in detail by Hamilton and Walton (1961). Wright (1954) combined a horizontal elutriator for the first stage of sampling with a soxhlet filter for the second stage.

In the case of bacterial aerosols the first stage of sampling may be effected by the 'pre-impinger', described by May and Druett (1953), in which the coarser droplets were collected in liquid contained in a glass bulb. By the use of liquid, the risk of death of bacteria secondary to drying was partly averted. But there was still some loss by the drying of droplets deposited on the glass above the level of the liquid. To overcome this a so-called ' tilting pre-impinger ' was developed (May, 1960). Wells (1933) described an apparatus for distinguishing

between dust-borne and droplet-borne bacteria. The former particles, which are coarser, were deposited on a sieve impactor (a circular plate with multiple round holes of specified diameter), prior to collection of the droplets on a slowly turning agar-covered surface (*see also* Du Buy and Crup, 1944).

Yet another device for the separation of coarse from fine particles is the cyclone separator, used by Harris and Eisenbud (1953) in conjunction with a filter system for the second stage of sampling. Large and small samplers (Harris, 1961; Hyatt and his colleagues, 1961) of this type are now commercially available. According to Dennis and his colleagues (1952) their size-separating characteristics closely mimic the separation in deposition between the upper and lower respiratory tracts in man.

Wolff and Roach (1961) described the ' conicycle ', in which a controlled centrifugal force is used to reject the coarser particles in a stream of the test air passing into the sampler at a controlled velocity. The finer particles which gain entrance to the sampler are in turn deposited on to a collecting surface by the same centrifugal action.

All the two-stage sampling methods considered above, however, are based on the assumption that there is a more-or-less sharp line between particles of hazardous and non-hazardous dimensions. This assumption was in the past warranted when interest centred on the essentially practical and clinical problems of pneumoconiosis research, but for the purposes of basic studies on the effects of inhaled particles it was desirable to have information on the full spectrum of particle size. Several types of multistage sampler have been developed to provide information of this kind. The ' cascade impactor ' described by May (1945) consisted of a series of slots of decreasing size mounted at decreasing distances above collecting plates, followed by a filter for the collection of the smallest particles. A modified version was described by Lippmann (1961). According to Davies, Aylward and Leacey (1951) the high velocity jets involved in sampling by this type of instrument may break up aggregates of finer particles which would have behaved, in practice, like coarse particles.

The ' conifuge ', devised by Sawyer and Walton (1950) is based on the same principle as the ' elutriator ', the gravitational force being increased by centrifugation. In this instrument air for sampling is introduced at the apex of a double-walled, hollow, rotating cone. The particles are deposited on the outer wall in descending order of size from the top to the bottom. On completion of sampling, glass slides set in the outer wall may be examined microscopically

for particle number. The instrument gives excellent particle size separation, but suffers the disadvantage that the sampling rate is low. It has been successfully used to measure the particle size distribution and concentration in cigarette smoke (Keith and Derrick, 1960). In the ' aerosol spectrometer ' (Goetz, 1957) the gravitation force is increased still further, with the result that particles with diameters of down to 0·04 μ may be collected.

New developments in the optical measurement of aerosols based on light-extinction and light-scattering were reviewed by Hodkinson (1966). Perhaps the greatest advantage of these methods is that they involve the minimum disturbance of the particles under measurement and, in some instances, optical measurement is the only method available.

In inhalation studies the measurements and the efficiency of controlling devices must be carefully and constantly checked. There are many examples in the history of aerosol science of discrepancies between the theory and practice (Davies, 1961), so that accurate measurement of exposure dose is only likely to be approached if information from a variety of types of measurement is pooled.

Fate of Inhaled Vapours and Particles after Deposition within the Respiratory Tract

The extent to which inhaled vapours are absorbed depends on numerous physical, chemical, physiological and pathological variables (Oberst, 1961; Ainsworth and Shephard, 1961). Solubility in tissue fluids and reactivity with tissue constituents determine the sites of absorption, the percentage absorbed and the mode of excretion. Uptake may occur at any point in the respiratory tract, including the nose and nasal sinuses, and cancer of the nose and ethmoid sinuses may be induced by the single inhalation of a volatile nitrosamine (Druckrey and his colleagues, 1967). Under conditions of continuous exposure a state of equilibrium is reached between the concentration of a soluble constituent in the inspired air and that in various parts of the respiratory tract but, where the exposure period is short and this state of equilibrium is not reached, factors such as depth of respiration and breath-holding time may markedly affect the extent to which an inhaled gas is retained (Boyland, McDonald and Rumens, 1946).

Some of the same problems apply to the retention of particulate matter, but whereas an unabsorbed gas may be simply expired, once a particle has been deposited anywhere in the respiratory tree, some means other than simple exhalation has to be employed by the body to eliminate it. Deposition in the nose and deposition in the larynx,

trachea, bronchi, bronchioles and alveoli present different problems. These are best considered separately.

Deposition in the Nose

The coarsest particles, together with a proportion of smaller particles, are arrested in the nose. By ciliary action these are transported in a stream of mucus back to the nasopharynx, there to be swallowed (or, in the case of man, expectorated). The pathways of ciliary streaming are fixed and unchangeable, according to Lucas (1933) and Hilding (1932). Negris (1958) gives references to the patterns of ciliary streaming in man and various other species. Sneezing may expel aggregations of particles in partly desiccated, viscous mucus via the anterior nares and the same may be achieved by nose-blowing. Under certain special circumstances, however, these methods of elimination are not sufficiently effective for the avoidance of hazard from inhaled particles, shown by the ulcers of the nasal septum in chrome workers (Schwartz and Sieke, 1930) and nasal cancer in nickel workers (Doll, 1958; Morgan, 1958). Partly because of the difficulties involved in the pathological assessment of the effects of inhaled materials on the nasal mucous membrane, particularly the need to decalcify specimens, there is relatively little information on the clearance of particulate matter from this part of the respiratory tract.

Deposition in Larynx, Trachea, Bronchi and Non-respiratory Bronchioles

There is evidence that, even in the damaged lung, the most effective means, quantitatively, for removing insoluble matter deposited in the trachea, bronchi and non-respiratory bronchioles is by the action of cilia and it has become customary to think of particles being cleared from these parts of the respiratory tree on an upward-moving mucus blanket, or escalator. This is said to consist of two layers of secretions, a thin one below in which the cilia actually beat and a thicker one above which moves as a result of the beating movement (Lucas and Douglas, 1934; Hilding, 1943). However, it is important to point out that in sections of normal trachea and bronchi no mucus escalator is visible; so that its presence is evidence of pathology. Mucus is produced in the bronchi both by the bronchial glands and by the goblet cells in the surface epithelium. Exposure to respiratory irritants such as sulphur dioxide (Reid, 1963), chlorine (Elmes and Bell, 1963), or tobacco smoke (Field and his colleagues, 1966), lead to hypertrophy of the mucus glands, an increase in the percentage of surface cells which secrete mucus, and an extension of the area of the respiratory tree

in which surface goblet cells are present. Goblet cells, which are not found in the bronchioles of normal persons, are seen in the bronchioles of those who are chronically exposed to respiratory irritants, or are suffering from chronic bronchitis. The mucus blanket is derived from the secretions of the hypertrophied glands and from the increased numbers of surface goblet cells, and its upward movement to the glottis is affected by the beating of cilia. Presumably, as the degree of respiratory irritation increases and, with it, the ratio of goblet cells to ciliated cells in the surface epithelium, the movement of the mucus blanket becomes slower. Elsewhere (*see* p. 58) the paralysis of cilia is considered separately.

The effectiveness of coughing as a clearance mechanism is dependent on the intact state of the cilial clearance system, since coughing only results in the removal of material from the larger bronchi and trachea. The mucus blanket is still required for the clearance of the more distal parts of the lung.

Clearance from Respiratory Bronchioles and Alveoli

Theoretically there are four possible fates for particles deposited on the truly respiratory areas of the respiratory system:

(1) Solution in body fluids and removal in blood stream.

(2) Removal on the mucus escalator.

(3) Removal in the lymphatics to the local lymph nodes, with or without subsequent passage into the blood stream.

(4) Sequestration within the lung, with or without subsequent lung tissue reaction.

There are two types of epithelial cells lining the alveoli—a larger, granular, phagocytic type and a smaller, non-phagocytic, endothelium-like cell (Macklin, 1954; Policard, Collet and Pregermain, 1956). According to Mendenhall (1963) the surface tension of the thin mucoid film which coats the alveoli varies from 46 dynes/cm during inspiration to 0 dynes/cm. during expiration. These variations in surface tension are associated with the alternate transudation and reabsorption of alveolar fluid during the respiratory cycle.

There is normally a slow turnover of the phagocytic moiety of the alveolar cells (Bertalanffy and Leblond, 1953). These, together with their burden of ingested particles, are desquamated into the alveolar lumen where they continue their phagocytic activities before ascending the mucus escalator. They may reach the bottom of the escalator either by their own amoeboid movements or, possibly, by one of the passive transport mechanisms postulated by Gross (1953) or Antweiler (1958). Tropism and increased respiratory activity

may also aid pulmonary clearance (Klosterkötter, 1957; Friedberg, 1960), though this is disputed.

Removal of dust particles from the lungs begins immediately after exposure and in the normal lung may be a remarkably efficient process. Inert particles, such as iron oxide or barium sulphate, deposited in the larger bronchi move upwards on the mucus escalator at a rate of between 1 and 2 cm per minute and the lungs may be more than 50 per cent cleared within a few hours (Cember and his colleagues, 1956). Particles and droplets small enough to reach the alveoli may also be removed rapidly, though mostly after ingestion (phagocytosis or pinocytosis respectively) by phagocytes. The number of these recoverable by flushing out the lungs with a balanced salt solution increases rapidly after exposure to fine dusts (LaBelle and Brieger, 1959). Cember and his colleagues (1956), from studies in rats, reported a 50 per cent removal of barium sulphate particles of mean size 1·45 μ diameter in two days of exposure and a 98 per cent removal by the eighth day. Over a range of exposure doses (up to 1 mg. in rats), the rate of clearance of inert carbon increases with dose; at higher doses (e.g. 10 mg. in rats) clearance is less efficient (LaBelle and Brieger, 1959). However, such rapid clearance does not always occur and, in some experiments, especially where non-inert dusts have been used or where test materials have been introduced into the lung by intratracheal instillation rather than by inhalation, some of the material has been retained for periods exceeding 12 months (Hatch and Gross, 1964). However, as pointed out elsewhere (*see* p. 43) chronic lung disease, which may well interfere with pulmonary clearance, is rife amongst laboratory rats and, unless stringent measures are taken to exclude such disease, it is inconceivable that it will not affect the outcome of long-term inhalation studies.

Ciliostasis

Factors, such as cold, dehydration, trauma, oxidant gases and viral infection, (Kilburn 1967) which interfere with ciliary activity and slow the movement of the mucus escalator, reduce the rate of pulmonary clearance. Some noxious gases, such as sulphur dioxide, chlorine or the oxides of nitrogen, temporarily delay pulmonary clearance. Influenza virus infections or areas of squamous metaplasia, on the other hand, have a more prolonged effect.

Numerous workers have studied the effects of gases and aerosols on ciliary activity with *in vitro* systems such as the frog's tongue or mammalian tracheal mucosa (Dalhamn, 1956; Tremer, Falk and Kotin, 1959; Hilding, 1961; Battista, 1962; Wynder and his col-

leagues, 1963; Kensler and Battista, 1963). Bernfeld, Nixon and Homburger (1964) pointed to the need for control of both temperature and humidity during all studies of ciliary inhibition, the absence of such controls partly invalidating many of the earlier studies on ciliostasis. Further information on the methods of measurement of ciliary activity is provided by Ballenger and his colleagues (1966), Rylander (1966) and Bernfeld (1966).

Sequestration of Inhaled Material within the Lungs and its Transport to Lymph Nodes

It is as yet impossible to predict the proportion of any inhaled material which will be eliminated by any particular route. In general, sequestration with the lungs or transport to local lymph nodes is more likely to occur when for any reason rapid elimination by the mucus escalator has not taken place. This state of affairs could arise if dust particles actually penetrate the intact alveolar wall (Hatch and Gross, 1964). However, the most significant factor seems to be the inertness or non-inertness of the inhaled material. Some dusts, e.g. quartz, excite a fibrogenic response within the lung and this effectively precludes subsequent elimination by the mucus escalator.

One of the most remarkable phenomena is the apparently specific tropism of asbestos dust to submesothelial tissues. In man this is manifest by the formation of mesothelial plaques and mesotheliomas. Roe and his colleagues (1967) showed that, following subcutaneous injection in the flank of CBA strain mice, asbestos of the three main types—crocidolite, amosite and chrysotile—was transported specifically to the submesothelial tissues of the pleura, pericardium and peritoneum. At these sites it gave rise to inflammatory, proliferative and neoplastic responses.

Measurement of Exposure Dose by Use of Radioactive Isotopes

Albert and his colleagues (1967) review the use of radioactive aerosols in the study of deposition and clearance of particles from the human lung. By the use of such methods these workers studied, not only the pattern of deposition of particles of various sizes in different parts of the respiratory tract (including the nasopharynx and the mouth), but also such problems as the effect of posture and exercise on the rate of bronchial clearance, the rate and pattern of intrapulmonary redistribution (e.g. passage of particles to the hilar lymph nodes), and the effects of respiratory irritants and of various lung diseases, on the deposition of particles and their clearance from the lungs.

ASSESSMENT OF THE EFFECTS OF INHALATION EXPOSURE

Disturbance of Respiratory Function

Many of the measurements of function which may be made on man, e.g. forced expiratory volume (FEV); forced vital capacity (FVC); forced expiratory time (FET); and peak expiratory flow rate (PEF) (Lloyd and Wright, 1963; Lal, Ferguson and Campbell, 1964; Gregg, 1964) are dependent on the ability of the subject to understand and comply with instructions. In laboratory animals the spectrum of possible measurements is much narrower. According to Alarie (1966), slowing of respiration as recorded on a body plethysmograph provides a sensitive index of the irritant properties of inhaled materials. Widdicombe (1963, 1964) refers to the measurement of ' the dynamic pressure–volume relationship ', or ' dynamic compliance ', by which he means measurements of volume changes with constant volume inflations, but in such studies it is necessary for histopathological assessment to be made as well. Since the measurements can be made only on anaesthetized animals and usually under highly unphysiological circumstances (Konzett and Rössler, 1940), they have but limited application in the evaluation of inhaled materials; their main value relates to the examination of drugs. Methods for the measurement of lung resistance to air flow have been reviewed by Comroe and his colleagues (1962) and Nadel and Widdicombe (1962). Boyd (1954) discussed methods for measuring the total output of mucus by the respiratory tract on tracheotomized anaesthetized animals and the use of radiography to measure effects on the lung is discussed by Kilburn (1960).

Immune Reactions of the Lung

According to Rose and Phills (1967) the lung is capable of three types of immune response: allergen–reagin reactions, antigen and precipitating antibody reactions and delayed-type hypersensitivity reactions. In the first type the production of chemical mediators which induce a bronchial-asthmatic response is stimulated by an allergen–reagin reaction. Histamine is only one of these mediators; apparently a more slowly reacting substance (SRS) is also involved (Brocklehurst, 1956, 1960). The same or similar substance is implicated in guinea-pig anaphylaxis. In man, the allergen-reagin type of response is limited to individuals of the ' atopic ' type (Coca and Cooke, 1923) and is characterized by tissue injury, release of histamine, SRS, and possibly other mediators. According to Rose and

Phills (1967) most of the allergens involved in this type of reaction are proteins of molecular weight ranging from 3000 to 40,000. They tend to be present in the air as particles ranging from 10 to 100 μ (e.g. pollens) and are accordingly deposited almost entirely in the nose, though in mouth breathers they may reach as far as the larynx, trachea, and larger bronchi.

Antigen and precipitating antibody reactions may be caused by a variety of fungal spores, e.g. *Aspergillus fumigatus*, *Thermopolyspora polyspora* (farmer's lung), mould-contaminated bagasse fibre (sugar cane lung), *Coniosporium corticale* (maple bark disease). Other probable examples according to Rose and Phills (1967) are ' bird breeders' lung ' and a respiratory syndrome observed in mushroom growers. The fungal spores which give rise to these reactions are of much smaller particle size than the pollens and dusts usually involved in the allergen–reagin types of reaction. Thus the spores of *T. polyspora* are only 1 μ diam. and can penetrate readily to the distal parts of the lung. The response to the inhalation of such spores may be of the simple allergen–reagin type described above. Alternatively a hypersensitivity response involving the production of precipitating antibodies, pulmonary infiltration, and eosinophilia may occur (Pepys and his colleagues, 1959). The latter is characterized clinically by influenza-like attacks.

Tubercular-type hypersensitivity reactions are seen in response to the tubercle bacillus, certain fungae, histoplasma and coccidioidomycosis. The response is characterized by the development of epithelioid granulomas in the lung and delayed-type hypersensitivity skin reactions to antigens prepared from the causative organism.

Most of the immune reactions which involve the respiratory system of man are associated with exposure to vegetable or microbial agents, but some chemical agents may induce similar responses. Thus chlorogenic acid may induce asthmatic attacks in atopic individuals and there is evidence that pulmonary berylliosis is a delayed-type hypersensitivity phenomenon (Curtis, 1951; Morris and Peard, 1963). Similarly, according to Balchum and his colleagues (1965), the inhalation of nitrogen dioxide may lead to the appearance in the serum of guinea pigs of a lung tissue antibody in high titre. Delayed-type hypersensitivity may also be involved in silicosis, according to Powell and Gough (1959).

In the present state of knowledge it is not possible to predict from screening tests in animals which chemical or other agents are liable to produce adverse immune reactions in the human lung, though wherever an immune mechanism is suspected, experimental studies will often assist in its elucidation.

Pathological Effects on Respiratory Tract

The method used for killing an animal is relevant to the histopathological assessment of the results of inhalation exposure. Any form of slow asphyxial death is liable to cause congestive changes and petechial haemorrhage in the lungs. For that reason, killing by overexposure to ether, for example, is unsatisfactory. On the other hand, good results may be obtained by starting ' necropsy ' on the unconscious animal which has received an overdose of nembutal.

The best method for examination of the nasal mucosa and air sinuses is to remove the skin of the head and the brain, fix the remaining part of the head, decalcify and cut transverse sections through the nasal region at several levels.

Fixation of lung tissue may be achieved by flooding the respiratory tree with formalin vapour or with formol saline from the trachea. In this case it is desirable to avoid inflow pressures which exceed 25 cm. water. Fixation by formalin vapour may be achieved by placing the lung in a box with the trachea protruding. When suction is applied to the space between the lung and the wall of the box, formalin vapour is drawn into the lung. In the author's view the results of fixation by this method do not justify the extra difficulties involved. Alternatively, good fixation may be obtained by the intra-arterial or intravenous infusion of fixative via the pulmonary vessels.

Where interest centres on the mucosa, the so-called Swiss-roll technique (Knudtson, 1956) may be helpful in the case of larger animals. However, the small diameter of the trachea of the rat and the mouse render the technique difficult to apply. For these species Innes, Garner and Stookey (1967) recommended the removal and fixation of the trachea and lungs in one piece, followed by slicing in a plane such that both main bronchi are sectioned longitudinally in continuity with the trachea. Sections prepared in this way show the mucosa from the larynx to the bronchioles to good effect. Such sections are suitable for measuring the ratio of mucus goblet to ciliated cells in the surface epithelium. Lamb and Reid (1967), on the other hand, point to the advantages of making a sagittal section of the trachea since this enables the examination of the anterior part of the trachea where mucus glands are especially numerous. They also recommend sectioning of the left lung of the rat and the mouse on a sledge microtome with an adjustable block holder as this enables one to obtain a section which includes the whole of the main bronchus and its principal branches. Good preparations of the whole right lung are impossible to obtain because of its subdivision into four lobes in different planes; if it is necessary, separate sections should be taken from each lobe.

In the case of species which possess bronchial mucus glands the methods of measurement devised by Reid (1960) are applicable. The two most important of these are the gland–wall ratio (i.e. the ratio between the maximum depth of the mucus gland to the distance between the basement membrane and the tracheal or bronchial cartilage), and the acinar count (i.e. the number of acini per uniform microscopic field in a mucus gland area). Under conditions where mucus secretion is active, acinar hypertrophy is accompanied by a reduction in the acinar count.

Other measures of ' irritation ' include observations on the thickness and intactness of the basement membrane, on the thickness of the basal cell layer (which is conspicuous in larger animals, such as man, donkey, and dog, but not in rats and mice), on the presence and extent of peribronchial infiltration with inflammatory cells of various types including eosinophils and on the presence and extent of squamous metaplasia, cellular atypia, carcinoma *in situ* and frank neoplasia. Gross observations may be made on the accumulation and distribution of inspired particulate matter, but for most purposes it is desirable to supplement these by the chemical estimation of residues or by tracer studies and autoradiography. Effects on cell population kinetics may be measured by the usual technique of administration of tritiated thymidine, with or without the subsequent administration of Colcemid.

A comparison of wet weight and dry weight may provide a measure of oedema, but it is essential that the method of killing is rigidly standardized. Control of the blood content of the lung is especially important. This may be achieved by exsanguination, or by tying off the vessels at the base of the heart whilst it is still beating.

Pulmonary macrophages may be viewed in sections of the lungs after fixation, or recovered quantitatively from the lungs immediately after death by flushing out with a balanced salt solution.

Assessment of the degree of pulmonary fibrosis is possible by the methods of Widdicombe (1963, 1964) referred to above, though such measurements should always be checked by histopathological examination for which purpose staining of sections with orcein and van Gieson is recommended.

Corn and Burton (1967) stress the need to define the term ' irritant ', whether this should be applied to something which leads to inflammatory tissue response, or to changes which involve increase in airway resistance or other adverse change in pulmonary function. Thus aerosolized histamine leads to the latter but not to the former.

Further, the effect of mixtures is not predictable from knowledge of effects of individual substances, e.g. in air pollution (Corn and Burton, 1967).

There is evidence that the lungs may become tolerant to respiratory irritants. Whereas on the first occasion exposure to nitrogen dioxide at a concentration of 30 ppm for two hours significantly increased the rate of breathing for a period of two days, subsequent similar exposures were easily tolerated (Henschler, 1962). The same phenomenon of tolerance has been seen in the case of ozone (Kleinfeld and Giel, 1956; Challen, Hickish and Bedford, 1958). However, as pointed out by Fairchild (1967), the fact that tolerance may develop to the acute effects of irritants does not imply that increasingly serious chronic damage will not occur.

Fairchild (1967) reviews ways in which the effects of certain respiratory irritants may be reduced. Thus hormone-administration and/or endocrine ablation enables rats to survive ozone poisoning (Fairchild, 1963); mice can be protected from the effects of ozone and nitrogen dioxide by the administration of sulphur compounds (Fairchild, Murphy and Stockinger, 1959), ascorbic acid (Matzen, 1957), combinations of promethazine and aspirin (Dixon and Mountain, 1965), or by the inhalation of oil mists (Wagner, Dobrogorski and Stockinger, 1961). In all cases there is a reduction in oedema.

Combined Effects of Infectious Agents and Chemicals

In a discussion of the effects of viral infections on the respiratory system, Hornick (1967) stresses the need for more information. The pulmonary damage left after infection with influenza or other viruses may be permanent. Viral infections, which in normal individuals tend to be confined to the upper respiratory tract, tend to give rise to acute bronchitic reactions in patients with chronic bronchitis (Ehrlich, 1963). Changes in respiratory function have been followed over long periods by Bocles, Ehrenkrantz and Marks (1964) in patients with varicella pneumonia and by Berven (1962) in patients with atypical pneumonia.

Pathological Effects on Other Organs and Tissues

Where an inhalation test is intended as a screen for general toxicity it is essential that necropsy is as complete as in any feeding study of a food additive or any toxicological study of a drug. Clearly soluble materials may pass from the nasal mucosa or from the lung to other parts of the body, and there are numerous examples of agents which exhibit remote effects in the absence of local effects.

Insoluble materials such as asbestos may also travel to distant sites (Roe and his colleagues, 1967), so that it is unwise to depart from a routinely extensive necropsy procedure on the basis of preconceived ideas of likely non-effectiveness or non-transport from the lungs.

GENERAL CONCLUSIONS

Of all the possible routes by which toxic materials may enter the body, there is least reliable information about inhalation. Theoretically, recent rapid advances in the basic knowledge of the physics of aerosols and particle clouds, of methods for their generation and of respiratory anatomy and physiology have made controlled inhalation studies on laboratory animals a possibility. In practice, few institutions have the equipment and resources necessary for such studies. In particular there is a serious shortage of facilities for long-term inhalation experiments.

In the present article some of the basic requirements for a research facility for inhalation studies have been discussed in the light of the problems involved. These basic requirements include properly designed inhalation chambers, the means for the generation of standard aerosols and particle clouds, instruments for monitoring exposure, disease-free genetically-defined animals, the microbiological control of animals under experiment, methods for the measurement of lung function, and adequate facilites for the histopathological evaluation of the effects of exposure and for radioactive isotope tracer work.

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