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13. ABSTRACT

Acute exudative or hemorrhagic lesions were seen in the lungs of monkeys, dogs, rats and mice exposed to high concentrations of ozone, nitrogen dioxide and oxygen, while subacute proliferative pulmonary lesions were best demonstrated in monkeys exposed to high concentrations of oxygen and surviving the early exudative phase of the toxic response. The chronic granulomatous response was best illustrated in the lungs of dogs exposed to beryllium-containing dusts and allowed to recover for 30-36 months.

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PATHOLOGICAL RESPONSE OF THE LUNG TO CERTAIN INHALED IRRITANTS

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INTRODUCTION

The Air Force is vitally interested in providing a safe and habitable environment for its space crews and associated ground-launch crews. Since much of the potential danger is likely to be from inhaled toxic substances, the Toxic Hazards Division, 6570th Aerospace Medical Research Laboratores, has studied intensively various inhaled materials for the past few years. These studies have provided unusual amounts and types of pathologic material where the animal lung has been the target organ. It is the purpose of this report to review and categorize the classical pathologic responses of the lung according to the chemical irritant, the dose of that irritant, and the species of animal concerned.

MATERIALS AND METHODS

Most of the inhalation-toxicology experiments to be discussed were conducted in the Toxic Hazards Research Unit (THRU) which was designed by Dr. Anthony A. Thomas, Director of the Toxic Hazards Division. The four Thomas domes in the THRU are unique dynamic flow experimental altitude chambers in which the environmental conditions are automatically controlled to narrow ranges. [16] Pressures can be maintained at prescribed levels from 5 to 14.7 psia and oxygen concentrations can be maintained at levels from 20 to 100 per cent. Air flow rates usually average 20 cu ft/min (approximately 500-650 liters/min). The contaminant is generated and metered into the incoming air or oxygen in carefully monitored concentrations. Temperature remains at about 72°F (22.2°C) and the average relative humidity at about 50% with only temporary fluctuations, usually during the entry and cleaning procedures. When dome pressures exceed 720 mm Hg, pressure is reduced to 720 mm Hg when the dome is entered. Entries are made through an airlock daily to care for the animals and as often as required to remove dead animals. The usual complement of animals for one experiment includes 4 monkeys, 8 dogs, 50 rats and 40 mice. There are variations in these numbers based on the requirements of a specific experiment. Dogs and monkeys are fed once daily, rats and mice have food accessible continuously, and all animals have water adlibitum.

Dead animals are removed from the domes as soon after death as possible. Dogs and monkeys that survive the exposures are euthanatized with overdoses of an intravenous barbiturate anesthesia while rats and mice are euthanatized with overdoses of ether. Necropsy examinations are made on all animals and weights of the heart, lung, liver, kidneys, and spleen (also on brains of monkeys) are recorded. Tissues from all organ systems from monkeys and dogs are preserved in buffered formalin while only selected tissues (heart, lung, liver, kidney, and spleen) are saved from rats and mice. Lungs are sometimes inflated by tracheal perfusion with the formalin fixative. Fixed tissues are embedded in paraffin, sectioned at 5 microns, and stained with hematoxylin and eosin. Special stains are used to demonstrate particular elements or structures.

Exposures of dogs to beryllium-containing dusts were done by the Atlantic Research Corporation under Air Force contract. [10] They were exposed to an average concentration of either 115 or 120 mg/m^3 for 20 minutes and held in a vivarium for 30-36 months before they were euthanatized and tissues processed as above. Samples of the lung for electron microscopy were taken from two dogs.

RESULTS

The pathologic responses of the lung to the irritants studied in the Toxic Hazards Research Unit have been divided into the acute exudative

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The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research. The research reported in this paper was conducted by personnel of the Aerospace Medical Research Laboratories, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio 45433.

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hyperplasia of the alveolar epithelium. Mild hypertrophy of the alveolar lining cells in addition to mild edema of the septal tissue was seen as early as the 5th day of exposure. Residues of the earlier exudative phases of oxygen toxicity were still evident and consisted of resolving fibrin with entrapped inflammatory and desquamated epithelial cells.

At the end of the two-week exposure, the interstitium of the septae was greatly thickened due to collagenous as well as reticular fibers. Only modest increases in vascularity accompanied the proliferative changes and there was no congestion or hyperemia. The alveolar lining cells were enlarged and hyperplastic, often appearing to be several cells thick. Desquamated alveolar lining cells appeared in the alveoli in large numbers along with a few mononuclear phagocytes(13).

Chronic granulomatous lesion

Dogs exposed to beryllium-containing dusts and held for 30-36 months developed widley scattered granulomatous foci from which beryllium was identified by microemission spectrography(10). Rabbits similarly exposed did not develop these pulmonary lesions.

Multiple, discrete focal granulomas were scattered throughout the lungs of 4 of the 8 exposed dogs. The larger granulomas measured up to 500 microns in diameter with occasional lesions exceeding 1 mm. These lesions were not easily discernible on gross examination. The smaller granulomas consisted of a few centrally located macrophages containing small black particles superimposed on a yellow cytoplasm. Surrounding these cells was a mixture of lymphocytes and occasional plasma cells that tended to be located in the periphery of the granuloma. The larger granulomas were composed principally of large mononuclear macrophages also containing the dark particulate material. Lymphocytes tended to form clusters in the interstices between these particulate-laden groups of macrophages(10) Connective tissue elements were more evident by electron microscopy than by light microscopy. The increase in collagen in the granulomas was directly associated with the interstitial deposits of beryllium(14).

DISCUSSION

The incidence of acute pulmonary lesions produced by either ozone or nitrogen dioxide is directly dose dependent in the higher dose levels (8 mg/m^3 and higher for ozone and 36 mg/m^3 and higher for nitrogen dioxide). Mortality and the incidence of the acute lesions are very low when monkeys, dogs, mice and rats are exposed to Threshold Limit Values for 90 days (TLV for ozone is 0.2 mg/m^3 and for nitrogen dioxide 9.4 mg/m^3) (15).

Acute pulmonary lesions in man due to oxygen toxicity have been reported (3,9). One fatal human case arising as a result of hyperbaric oxygen therapy had pulmonary lesions including intra-alveolar hemorrhage, fluid transudation, and intra-alveolar fibrinoid or hyaline membranes(3). In a study of autopsied human patients, capillary congestion and proliferation were seen in patients receiving oxygen therapy for as little as 2 days (9).

Subacute proliferative lesions nearly identical with those described above in monkeys have been reported recently in human patients receiving oxygen therapy and artificial ventilation, the so-called "respirator lung syndrome" (7,8). One of these authors also used the terms exudative and proliferative to describe the two characteristic phases of the disease(7). The same author correlated these changes with the prolonged use of the ventilator delivering a high concentration of inspired oxygen and states that inspired oxygen concentration should be monitored and reduced as soon as arterial blood-gas measurements show that the reduction can be accomplished safely. In a recent study in our laboratory, monkeys were exposed

or hemorrhagic, subacute proliferative and the chronic granulomatous types. This classification is at times arbitrary when the lung is recovering from the acute stages of oxygen toxicity and is beginning to develop the subacute proliferative stage.

Acute exudative or hemorrhagic lesion

Acute exudative pulmonary lesions occurred in all species exposed to high concentrations of oxygen. These lesions characteristically were seen in animals that died after 2-4 days exposure (Table I). Their lungs were firm and red to bluish red, and collapsed if the exudation of pleural fluid was extensive. As much as 10 ml of pleural fluid was withdrawn from 150 gram rats.

Microscopically, there was marked capillary congestion, alveolar edema and varying amounts of hemorrhage into the alveoli. Interstitial inflammation became evident in animals surviving more than 3 or 4 days. (12)

In an ultrastructural study of the lungs of rats exposed to high concentrations of oxygen, interstitial edema appeared after two days exposure suggesting damage to the endothelial cells. A morphometric analysis showed a significant reduction in the average thickness of endothelium only after three days. At this time ultrastructural changes in the endothelium were pronounced, such as darkening of the cytoplasm of endothelial cells in which the organelles could still be recognized even though the cell was detached from the basement membrane. Final stages in endothelial cell destruction were characterized by capillaries bounded exclusively by basement membranes which were in direct contact with erythrocytes. There was a lack of blood plasma while precipitated fibrin strands were seen near the basement membrane. (4)

The acute hemorrhagic type of lesion is seen in all species exposed to high concentrations of ozone (Table II) and nitrogen dioxide (Table III). The lungs of these animals were red, wet and heavy with little increase in pleural fluids. The only marked difference in gross appearance was the patchy distribution of the hemorrhage in the lungs of animals exposed to ozone.

Microscopically, there was widespread hemorrhage and alveolar edema with varying amounts of congestion. The edema was usually centrally located in the lobe and did not always extend to the periphery of the lung.

A marked difference in species susceptibility to ozone and nitrogen dioxide was evident between monkeys and dogs. Monkeys were more resistant to ozone and more susceptible to nitrogen dioxide than dogs. But regardless of the dose, if the animal died during the 14-day experiments, its lungs invariably had the acute hemorrhagic lesion. There was an increase in lung weights with increases in dosage as illustrated for ozone in Table IV.

Subacute proliferative lesion

If the animal withstood the high oxygen levels for 4-5 days, it went on to produce the subacute proliferative response that was progressive up through the 16th day. The subacute proliferative response was not as marked in dogs, rats, and mice as in monkeys and was best typified in latter species when exposed for two weeks to high concentrations of oxygen. The lungs of these monkeys were nearly bloodless and tan or yellowish gray with an almost dry surface. At this time, the weight of the lungs was about three times the weight of comparable control lungs. They were quite firm and sank in the fixative.

Microscopically, these lesions consisted of variable amounts of proliferation of the pulmonary interstitium and hypertrophy and still later

to 100% concentrations of oxygen at ambient pressures to correlate blood gas data with morphologic changes. This research is not yet complete.

Considerable controversy rages over the validity of comparing beryllium-induced granulomas in animals with beryllium disease in man[2]. The morphologist says that the lesions are not comparable and the immunologist points out that beryllium disease in man is so sporadic among those exposed that there must be an immunological process involved that has not been reproduced in animals. The anatomic pathologist readily admits that beryllium disease in man cannot be diagnosed solely upon the morphological characteristics and gives a positive diagnosis, in most cases, only after an exposure to beryllium has been confirmed and a chemical analysis of the patient's lungs shows the presence of significant amounts of beryllium. Radiological evidence is helpful as is a documented history of progressive lung disease unattributable to any other cause. The suggestion has been made that beryllium-induced lesions in dog-lungs are similar to the early stage of the disease found in humans and if the dogs were allowed to live long enough they might also develop the diffuse granulomatous disease seen in man[11]. This contention is supported by the fact that when humans with clinical cases of berylliosis die of other causes, their pulmonary lesions due to beryllium are quite similar to those seen in the dog-lungs. From the immunologic standpoint, there has been very little evidence that the disease has an immunologic basis even though considerable effort has been expended in this area.

Until recently, the cause-effect in beryllium disease has been by inference since there was no reliable way of detecting beryllium in the induced lesion by any means, histochemical or otherwise. The only connection was the increase in average beryllium content of the lung of an individual with the disease. This average content was measured by wet chemical methods such as conventional spectrography, fluorometry, or other methods which destroyed the anatomic relationships of the tissue. With advent of laser technology, it was finally possible to sample microscopic areas of tissue using microemission spectrography. This method of analysis was used to definitely associate beryllium with experimentally-induced granulomas in dog-lungs[10]. The same method has subsequently been used to successfully analyze lung tissue from human cases of berylliosis. In an effort to semi-quantitate the results of this work, it was found that there must be a concentration of at least 50 ppm of beryllium in the sample analyzed to be detected by microemission spectrography[1]. This concentration seems rather high but it has been demonstrated that in chronic cases beryllium is usually concentrated in microscopic sites where these high levels could easily occur.

SUMMARY

Acute exudative or hemorrhagic lesions were seen in the lungs of monkeys, dogs, rats and mice exposed to high concentrations of ozone, nitrogen dioxide and oxygen, while subacute proliferative pulmonary lesions were best demonstrated in monkeys exposed to high concentrations of oxygen and surviving the early exudative phase of the toxic response. The chronic granulomatous response was best illustrated in the lungs of dogs exposed to beryllium-containing dusts and allowed to recover for 30-36 months.

TABLE I

Total pressure of oxygen (600-750 mm Hg) that produce death and acute hemorrhagic or subacute proliferative pulmonary lesions in animals exposed for periods up to 16 days[6].

Pressure	Monkeys	Dogs	Rats	Mice
760	3/4*	4/4	55/100	40/40
720	0/4	4/4	41/92	39/40
695	1/4	8/8	1/50	34/40
650	2/4	2/8	1/80	21/40
600	0/4	1/8	0/80	1/40

* 14-day exposure

TABLE II

Ozone concentrations at 260 and 700 mm Hg that produce death and acute hemorrhagic pulmonary lesions in animals exposed for periods up to 14 days [5].

Absolute Pressure (mm Hg)	Average Concentrations (mg/m^3)	Mortality No. dead / No. exposed			
		monkeys	dogs	rats	mice
750	0	-	-	0/50	0/40
700	8	2/4	5/5	50/50	34/40
700	15	1/4	8/8	50/50	40/40
260	0	0/4	0/4	3/50	3/80
260	8	0/4	2/8	45/50	33/40
260	15	0/4	6/8	-	-

TABLE III

Nitrogen dioxide concentrations at 260 and 700 mm Hg that produce death and acute hemorrhagic pulmonary lesions in animals exposed for periods up to 14 days(5).

Absolute Pressure (mm Hg)	Average Concentration (mg/m^3)	Mortality No. dead / No. exposed			
		monkeys	dogs	rats	mice
750	0	-	-	0/40	0/40
700	36	4/4	0/5	5/50	2/40
700	89	4/4	8/8	50/50	40/40
260	0	0/4	0/4	0/40	0/40
260	38	2/4	0/8	3/50	0/40
260	81	4/4	7/8	37/50	40/40

TABLE IV

Lung weights (per cent of body weight) of animals exposed to ozone for periods up to 14 days at 5 psia in 100% O_2 (5).

Concentration mg/m^3	Rat		Beagle		Monkey
	male	female	male	female	female
1.9	0.8	0.9	1.0	1.2	0.8
4.2	1.2	1.0	1.5	1.2	0.7
8.0	2.1	2.4	2.9	2.0	1.0
15.4	-	-	3.4	-	1.4

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LOCOWEED TOXICITY IN HORSES*

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Locoweed poisoning or "locosim" is a disease of horses, cattle, and sheep caused by excessive and continued consumption of certain species of plants belonging to the genera Astragalus and Oxytropis. The first published report of locosim was in 1873 (5). Since that time the syndrome has been periodically seen throughout the Western United States.

Outbreaks may be caused by at least 13 different species of plants belonging to the groups Astragalus and Oxytropis. The plants stay green all year and thus animals tend to eat them when the weather is dry and other forage is scarce. The plants are also toxic when dried.

\ poisonous principle was first isolated in 1936 and called locoine (3). Further studies have characterized it as an alkaloid, but it has not been fully identified and its chemical nature is still uncertain (1, 2, 4). Other chemical substances called earleine and pinite have been isolated (8, 9).

Locoweeds are distasteful but all livestock may develop a craving for them, especially if salt is not available. Horses seem to become accustomed to the plants more readily than do cattle and sheep. Animals poisoned on locoweed show a preference for the plants and recovered individuals may retain a craving for them.

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