INVESTIGATION OF ALVEOLAR MACROPHAGES FROM RATS EXPOSED TO COAL DUST*

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Abstract—Rats were exposed to the inhalation of coal dust from either Utah (low prevalence coalworkers' pneumoconiosis (CWP)) or Pennsylvania (high prevalence CWP). Rats were sacrificed, the lungs removed and lavaged to obtain free cells. The number of alveolar macrophages recovered from rats inhaling these two coal dusts (exposures up to 4 months) was not remarkably different from the number recovered from rats inhaling filtered room air. This is in contrast to results obtained after intratracheal intubation of the dust. The capacity of the lavaged cells to phagocytize and kill bacteria decreased after exposure to either dust. The activity of certain enzymes also decreased.

INTRODUCTION

It is a widely accepted fact that the inhalation of coal dust contributes to the pathological and the physiological changes seen in the lungs of some coal miners. Coalworkers' pneumoconiosis can be defined as the accumulation of coal dust in the lungs and the response of tissue to its presence. HEPPLESTON (1947) has suggested that in essence the initial inceptive and crucial lesion in coalminers' pneumoconiosis is a mantle of macrophages burdened with coal-mine dust enmeshed in fibrous tissue around the respiratory bronchiole and lobular arteriole.

Since alveolar macrophages possess phagocytic properties, they are the first line of defence against inhaled particles which reach the deep lung, and are likely to be the first cells damaged by these particles. THOMAS (1965) reported that proteolytic enzymes are involved in inflammatory processes and cell injury. It is possible that macrophages which have engulfed coal particles may undergo regressive changes and eventually be lysed, thus releasing their load of lysosomal enzymes into the extracellular fluid. The enzymes may cause necrosis of vicinal cells and, according to VIGLIANI and PERNIS (1961), there is a proliferation and collection of new macrophages, fibroblasts, mast cells and pyroninophilic cells in the proximity of the injury.

Many hypotheses have been postulated to explain the fibrogenic process and most

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of them involve macrophages, either in the necrotic processes or in the production of factor(s) which bring the identical end result of fibrosis.

LABELLE and BRIEGER (1959) investigated the clearance of dust from the lungs as related to the number of free cells washed out. Their work, mainly from acute exposures, demonstrated that an increased number of alveolar macrophages enhanced pulmonary clearance of insoluble particles. BRAIN and FRANK (1968) described a reproducible technique for washing free cells (mostly alveolar macrophages) from the lung. Utilizing this technique, BINGHAM *et al.* (1968) demonstrated that the total number of cells recovered may be altered by the inhalation of certain metallic particles. Because it is apparent that coal-mine dusts have variable chemical and physical properties, it was of interest to expose animals to the same concentration of two coal dusts from mines where workers experience great differences in the risk of developing pneumoconiosis. Whether or not biological responses to two coal dusts would be different had not been investigated experimentally. The investigations to be described were designed to determine whether the inhalation of coal dust by rats altered: (1) the number of free cells washed from the lungs using a standard technique; (2) the type of cells as compared to those of control rats; (3) the phagocytic and bactericidal activities of the lavaged cells; (4) the activity of certain enzymes of the lavaged cells.

METHOD

Two bituminous coal-dust samples were provided by the National Institute for Occupational Safety and Health. Coal-dust samples were taken from a mine in Utah, where there is a low prevalence of coalworkers' pneumoconiosis, and from a mine in Pennsylvania that has a high prevalence of disease (LAINHART, 1969). The coal samples were micronized and supplied to us. The inhalation chambers were 27-in. stainless-steel cubes and the coal was fed into the chambers using a Wright dust feeder. The size distribution of the dusts in the chamber was determined using an Andersen impactor.

It should be pointed out that by this time we had chemically analysed the coals and were aware that the coals differed considerably in their content of metals. These data have been reported by NORD and BINGHAM (1973). Drs Pfitzer and Horstman (personal communication) found during the dust exposures that it was necessary to feed about 4 times as much Utah coal as Pennsylvania coal to obtain similar nominal concentrations and particle sizes.

Young adult albino rats (Greenacres control-flora) were subjected to the inhalation of coal dust at two concentrations, 2 mg/m^3 or 15 mg/m^3, for 6 h per day, 5 days per week and for periods up to 4 months. In addition, a group of aged rats (24 months old) were subjected to the inhalation of coal dust (2 mg/m^3). The size distribution of the two dusts in the chamber was determined using an Andersen impactor.

Cells were harvested from control and exposed rats using the procedure described earlier by Brain and Frank and modified by BINGHAM *et al.* (1968). The total cell count was determined with a Coulter Counter and periodically checked with a haemocytometer and a light microscope. The washings which remained were combined and centrifuged. Slides were made from some of the cell pellets for histochemical studies and the remaining pellet used for enzyme assays.

The phagocytic activity was determined by the method described by VASSALLO *et al.* (1973) using cells harvested from both control and exposed rats.

For comparison with inhalation studies, four groups of young rats, six per group, were subjected to intratracheal injection of Utah coal dust, Pennsylvania coal dust, carbon, or saline and the free cells harvested from the lungs after 24 h and counted.

RESULTS AND COMMENT

The size distribution of the two coal samples is presented in Table 1. The mass median aerodynamic diameter of Pennsylvania coal dust was 1.2 μm while the Utah coal dust was 1.6 μm.

The number of alveolar macrophages recovered from rats inhaling Pennsylvania or Utah coal dust at 2 mg/m^3 is presented in Fig. 1. Initially there was an increase in the number of cells recovered from about 50% of the young rats inhaling coal dust. The

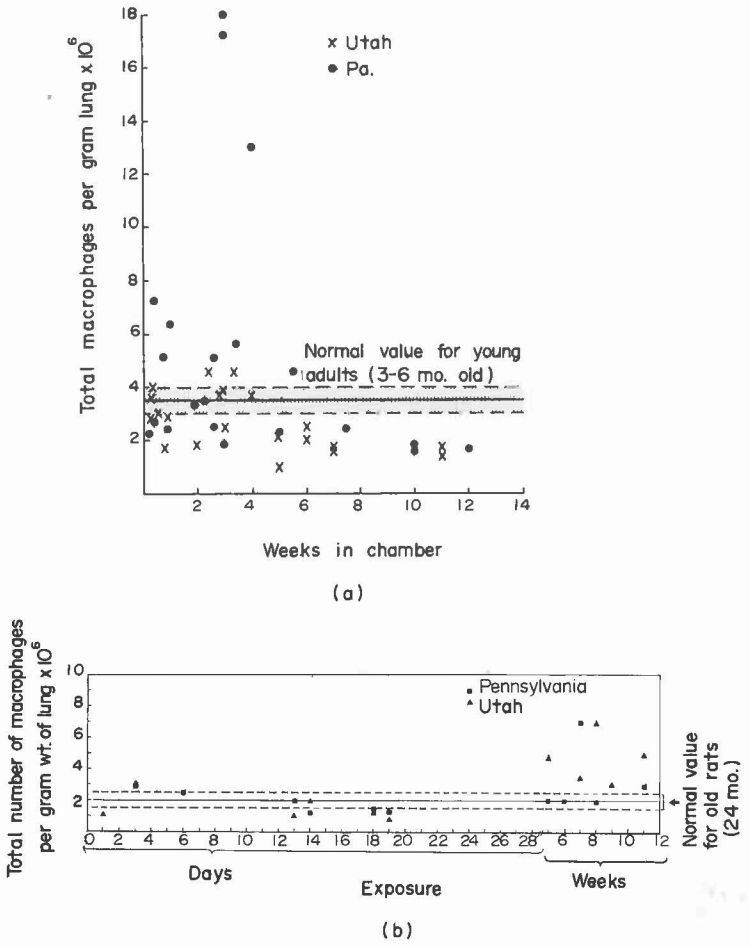

FIG. 1. Number of macrophages from rats exposed to coal dust (2 mg/m^3). (a) Young rats (3–6 months). (b) Old rats (24 months).

TABLE 1. PARTICLE SIZE DISTRIBUTION OF COAL DUST

Stage	MMAD[b] (μm)	Cumulative percentage of collected particulates[a]			
		Pennsylvania		Utah	
		total	respirable	total	respirable
1					
2	5.4	96.3	69.1	91.8	59.0
3	3.0	85.4	66.4	75.0	54.8
4	1.5	60.3	53.9	46.4	40.5
5	1.0	34.5	34.5	22.7	22.7
6	0.5	15.4	15.4	8.8	8.8
7	0.2	5.3	5.3	3.3	3.3

[a] Twenty-nine Andersen impactor samples; total volume sampled 102 m^3.
[b] Mass median aerodynamic diameter of particles collected on the stage.

majority of these animals inhaled Pennsylvania coal dust. In relation to the length of exposure the number of cells recovered from experimental rats gradually decreased to below control values. The number of cells washed from the old rats is about 2.0 $\pm$ 0.5 cells/g of lung $\times 10^6$. When old rats were subjected to coal dust, there was no difference between experimental and control data.

Since the number of cells did not increase markedly as had been reported by Brain in experiments with intratracheal injection, we performed similar injections. The results of these experiments are presented in Fig. 2. The initial increase is in agreement with Brain, who observed a great elevation in the number of macrophages washed out 24 h after intratracheal injection of carbon particles. Similar results are seen when coal dust, carbon, or saline (control) are injected intratracheally and the macrophages washed out 24 h later.

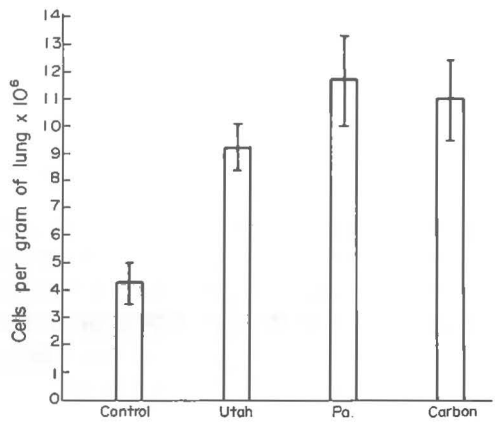

FIG. 2. Recovery of free cells 24 h after intratracheal injection of various particulates. Mean $\pm$ standard error.

The size of the cells washed from each experimental group exposed to inhalation of coal dusts is presented in Table 2.

TABLE 2. CELL SIZE DISTRIBUTION OF ALVEOLAR MACROPHAGES EXPOSED TO COAL DUST (2 mg/m³)

Exposure	Total number of cells counted	Cell size		
		<10 μm	10–20 μm	>20 μm
Control	200	138.0 ± 10.4	52.7 ± 7.1	8.7 ± 3.5
Pennsylvania	200	157.8 ± 8.8	43.8 ± 7.3	1.5 ± 0.6
Utah	200	175.5 ± 7.8	26.5 ± 7.6	0.3 ± 0.2

The activities of acid phosphatase, lysozyme, and β-glucuronidase were decreased in cells washed from rats exposed to inhalation of Pennsylvania or Utah coal dust. Esterase activity was increased in cells harvested from rats exposed to Utah coal dust; cells from rats exposed to Pennsylvania coal dust did not exhibit a significant increase in esterase activity. (Refer to Table 3.)

TABLE 3. ENZYME ACTIVITY* OF ALVEOLAR MACROPHAGES EXPOSED TO COAL DUST (2 mg/m³)

Enzyme	Enzyme activity		
	Control	Pennsylvania coal dust	Utah coal dust
Esterase	1.03 ± 0.04	0.98 ± 0.20	1.40 ± 0.18
Acid phosphatase	13.94 ± 4.45	3.93 ± 0.12	2.88 ± 0.12
Lysozyme	21.35 ± 6.29	6.41 ± 0.04	5.57 ± 0.67
β-Glucuronidase	0.13 ± 0.04	0.05 ± 0.01	0.03 ± 0.01

* Enzyme activity is expressed as specific activity = (μmoles of product released per mg of protein).

The capacity of the free cells harvested from coal dust exposed rats to ingest and kill bacteria is presented in Figs. 3 and 4. It can be seen that inhalation of either coal at either concentration resulted in a statistically significant decrease in the capacity of the macrophages to ingest and kill bacteria.

In conclusion then, even though these two coals differ in the production of disease in miners, and are distinctly different chemically in their content of metals, and are dissimilar in physical properties which became evident during the preparation of inhalation exposures—the differences in responses of macrophages have been similar. This does not mean that even small differences may be important over long periods of exposure or that we have even measured a critical biological response. A final point is that CHRISTIAN and coworkers (1973) in our Department have reported a significant difference in the toxicity of water extracts of these coals for cell cultures.

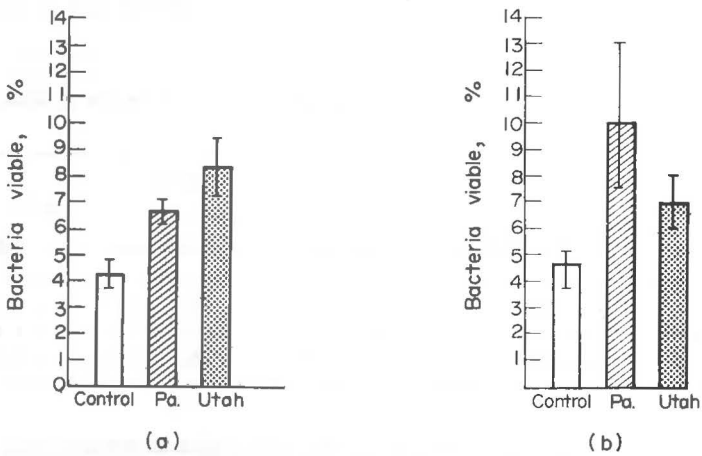

FIG. 3. Percentage of *Staphylococci* viable after phagocytosis by alveolar macrophages. Mean ± standard error. (a) 2 mg/m³ coal dust. (b) 15 mg/m³ coal dust.

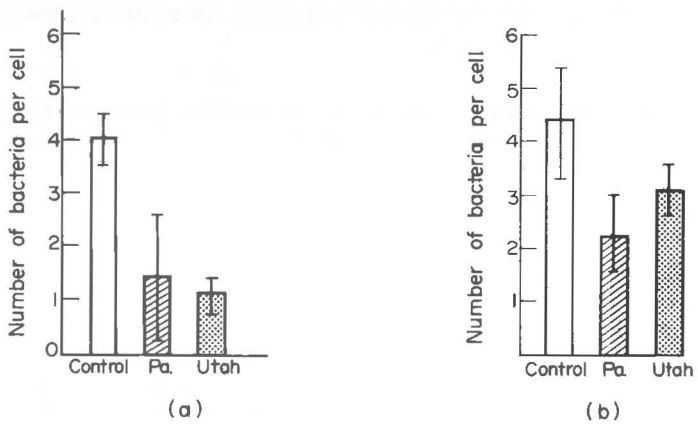

FIG. 4. Uptake of *Staphylococcus* by alveolar macrophages from rats. Mean ± standard error. (a) 2 mg/m³ coal dust. (b) 15 mg/m³ coal dust.

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DISCUSSION

P. COLE: How long after harvesting the alveolar macrophages did you perform the bacterial killing assay?

Dr BINGHAM: Within 30 min.

P. COLE: Did you use antibiotics in the culture medium during the assay? This is a technical point which has bedevilled work in this kind of system for many years (COLE, P. J. and BRONTOFF, J. *Nature, Lond.* 1975, **256**, 515–517).

Dr BINGHAM: Dr Vassallo, at the Cincinnati Veterans Administration Hospital, did these assays and has published details. Antibiotics were *not* used.

G. BOULEY: Can the fall in the phagocytic capacity of the macrophages of dusted rats in relation to the controls be explained by an increase in the immature macrophages in the dusted animals?

Dr BINGHAM: I think that is entirely possible. The macrophages do appear to be somewhat smaller and perhaps less mature. They have not, as it were, manufactured their bag of enzymes ready for delivery.

S. E. DEVIR: I wonder if the coal dust dispersed by the Wright dust feeder used in your inhalation study really represented the airborne particles the coal miner inhales. The drastic procedures of grinding, pressing, shearing and finally air blasting the coal particles, carried out with the Wright dust feeder, probably change the shape and the size distribution of the original airborne coal-dust samples, taken from the mines. Do you assume that these parameters (shape and size) will have no effect on the number of alveolar macrophages recovered from the lung of the rats in your experiments?

Dr BINGHAM: I think that is a valid point. These two batches of coal were supplied to us by NIOSH and they have, I believe, made some extensive measurements on the shape and physical characteristics of the particles. I cannot say more.

A. G. HEPPLESTON: I wonder whether the low macrophage recovery after coal inhalation may reflect the low rate of pulmonary accumulation of dust. When coal was given as a single intratracheal mass the greater stimulus presumably caused an increased outpouring of alveolar macrophages.

Dr BINGHAM: I would assume that is correct. However, we have washed out and counted macrophages from rats receiving higher exposures up to 15 mg/m^3 and have found an initial increase but not in every animal. Some animals do have apparently an outpouring of macrophages from the stimulus early in the exposure period. However, the number then returns to control levels. It is interesting that we have this levelling off.

J. M. G. DAVIS: This paper lays emphasis on numbers of macrophages washed from the lung after coal-dust inhalation. In the disease of pneumoconiosis, however, the major feature is the fact that many dust-containing macrophages become "fixed" within the lung. After 4 months' dust inhalation, therefore, the important cells may be those that cannot be washed from the lung.

Dr BINGHAM: You may be right, I do not have data on that.

T. L. OGDEN: Why was it that four times as much of one of the dusts as the other had to be dispersed to maintain equal concentrations? Was the dust obtained by grinding coal, or collecting airborne dust?

Dr BINGHAM: I think there were larger particles of the Utah coal that had to be separated out with a cyclone. The procedure was, of course, artificial because it is not that way in the mine. We were trying to have as close as possible the same nominal concentrations of the two coal dusts and as close as possible the same particle size distribution, to get at the question of whether or not there are chemical differences that could account for the difference in prevalence of disease.

H. AYER: There were no systematic measurements made of coal dust in American mines prior to 1969. Therefore, the relative concentration to which miners in the two areas were exposed is not known. Neither is it known whether the physical state of the dust in the coal mines of the areas resembles that dispersed into the dust chambers.

M. BUNDY: Do you know whether the Pennsylvania coal was anthracite from Eastern, or middle type from central or Western Pennsylvania?

Dr BINGHAM: It was from a mine near Johnstown, Pennsylvania.

M. BUNDY: Middle type. Further to Mr Ayer's remarks, the dust levels in Utah before 1969 were probably not as high as in Pennsylvania, although they were above the present standard level. Dust levels alone cannot, therefore, account for the difference in prevalence and incidence of CWP in the two regions; it can only be explained by differences in the coal.

Dr BINGHAM: And in people, I might add.

D. K. CRAIG: I cannot let the comments regarding the possibility of the Wright Dust Feed Mechanism (WDFM) modifying the nature of the dust particles go unchallenged. The scraper blade, revolving slowly, carves a 33-μm thick slice of dust off the dust pack and this is then dispersed with

compressed air. Providing the packing of the dust into the WDFM cylinder in the first place (at a pressure of the order of 250 kg/cm^2) does not modify the dust, and there is no evidence that it does, it is extremely unlikely that the coal dust would be altered by using the WDFM as the aerosol generator. In fact, the main problem with this device is that it is often not efficient enough in dispersing the dust.

In our studies, we have investigated the possibility that the Wright dust feeder might alter the nature of a softer powdered material than coal, namely talcum powder. We were unable to detect any such differences. Similar studies conducted elsewhere support our conclusions.

Dr BINGHAM: I am happy to hear that. I do know that the people concerned took much time and trouble to find the best method of dispersing the coal dust. They thought it was most appropriate at that time, but it has been 5 years since those experiments were begun.

INHALED PARTICLES

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