Study on Experimental Anthracosis

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INTRODUCTION

For the study of anthracosis, it is of importance to make it clear whether silicosis might be induced by coal dust. The massive silicotic fibrosis so frequent among colliers has been accepted as mainly due to the silica dust of the rocks that are always found in coal fields as sandstones and shalestones. Many recent authors, however, showed doubt about this theory, since the number of silicotic cases among the colliers detected by accurate medical examination with the latest excellent radiography is always apparently too high to justify such an assumption. But it has been difficult to ascertain the actual extent of silicotic lesions caused by coal dust alone, because the dust inhaled by coal-miners always includes both coal and rock particles.

The etiology of the silicosis so frequent among the colliers thus has not yet been adequately explained. The author of this paper, in view of this, has undertaken the experiments described hereunder for obtaining information on the effect of unmingled coal dust in the lung tissue. Indeed, there has been no report on experimental anthracosis produced by artificial inhalation of pure coal dust to date.

EXPERIMENTAL

Methods

In order to ascertain the actual effect of coal dust in causing lesions in the lung tissue over a long period of time, it was seemed better to rely on the method of inhalation rather than of intratrachale injection for producing experimental pneumoconiosis, though it costs a great deal of labor for a long time.

Animals: 30 rats, including males and females, about 4 months of age and weighing 200 g. on the average were used.

Coal dust: Coal from the Joban coal fields was used as raw material,
a kind of soft coal, containing 16.2% of ash. On chemical analysis, this ash was found to consist of 46.16% of SiO₂ (total silica), 5.06% of Fe₂O₃, 15.23% of Al₂O₃, 17.48% of CaO, 0.55% of MgO, 1.60% of K₂O and 8.54% of SO₃. It contained 4.24% of free silica as determined by N.A. Talvite's method. In the total coal dust, this content of free silica accounted for the mere 0.67%.

The samples of coal were ground to dust in a ball mill. In order to prevent mixing of silica fractions from the pot and the balls, the inside of the pot was coated with a rubber film and lumps of coal were used instead of the balls.

Using this coal dust, the rats were exposed to a high concentration of coal dust 12,000 to 15,000 particles per cc. of air in the dusting cabinet. Upon testing, the coal dust in the dusting cabinet was found to contain 82% of particles below 3 micron in diameter, particles of over 5 micron being only rarely found. The dust blow was given for 5 hours each on six days every week, except in summer, when the blow was limited 4 hours each on three days every week.

Duration of experiments: The experimental animals were sacrificed at about one months' intervals and the series of experiments extended over a total of 802 days.

Histopathological technique: Routine necroscopy was carried out on all the sacrificed animals as well as those found dead. The lungs were gently extended by injecting about 10 cc. of 10% formal saline into the trachea exposed at the neck, then extracted with the tied-off portion of the trachea and fixed in 10% formol saline, for more than 4 days. The blocks were then treated with hematoxilin-eosin and by Mallory-Azan method or silver impregnated by Bielschowsky's method or with Van Gieson's stain.

Results

The pathological changes produced in the lungs of the animals have been assessed according to Belt and King's classification, into the 5 grades of fibrotic lesions: Grade 1, loose reticulin fibers without collagen formation; Grade 2, compact reticulin fibers with or without collagen formation; Grade 3, slightly cellular but almost entirely collagenous; Grade 4, wholly collagenous and completely acellular, and Grade 5, acellular, collagenous and confluent.

The pathological grading obtained, together with the number of days of survival and the modes of death of the animals are summarized in the following Table I.

Gross appearance of the lung: At the beginning, or within 100 days of the experiment, the naked-eye appearance of the lungs and the pleura of the rats exposed to coal dust inhalation showed no difference from those
TABLE I

Assessment of Fibrosis in Sections of Lungs of Rats to Inhaled Natural Coal Dust

<table>
<thead>
<tr>
<th>Days of survival</th>
<th>Days of inhalation</th>
<th>Hours of inhalation</th>
<th>Mode of death</th>
<th>Productive alterations of lung tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade of fibrosis</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>42</td>
<td>K</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>12</td>
<td>70</td>
<td>K</td>
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<td>K</td>
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<td>0</td>
</tr>
<tr>
<td>84</td>
<td>62</td>
<td>332</td>
<td>K</td>
<td>0</td>
</tr>
<tr>
<td>92</td>
<td>64</td>
<td>344</td>
<td>D</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>68</td>
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<tr>
<td>128</td>
<td>82</td>
<td>482</td>
<td>K</td>
<td>0</td>
</tr>
<tr>
<td>172</td>
<td>124</td>
<td>726</td>
<td>D</td>
<td>1 Spot</td>
</tr>
<tr>
<td>260</td>
<td>161</td>
<td>958</td>
<td>K</td>
<td>1 Nodular</td>
</tr>
<tr>
<td>305</td>
<td>161</td>
<td>958</td>
<td>K</td>
<td>2 Nodular</td>
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<tr>
<td>421</td>
<td>186</td>
<td>1156</td>
<td>D</td>
<td>2-3 Nodular</td>
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<td>423</td>
<td>186</td>
<td>1156</td>
<td>K</td>
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<td>523</td>
<td>186</td>
<td>1156</td>
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<tr>
<td>608</td>
<td>259</td>
<td>1554</td>
<td>K</td>
<td>4 Nodular Asterisk</td>
</tr>
<tr>
<td>708</td>
<td>259</td>
<td>1554</td>
<td>K</td>
<td>4 Nodular Asterisk</td>
</tr>
<tr>
<td>802</td>
<td>259</td>
<td>1554</td>
<td>D</td>
<td>4-5 Nodular Asterisk</td>
</tr>
</tbody>
</table>

K: Killed; D: Died.

of normal rats. On the 172nd day, small pin-head form black spots were found on the surface of the upper part of the lungs, which grew steadily in size and consistency as the experiment went on. These nodular spots were usually rough and irregular in form. Finally, the spots fused together and were scattered all over the surface of the lung.

The presence of a standing infectious process in the lung, however, facilitates the activation of the coal dust inhaled, so that fibrotic foci develop earlier in greater abundance. In the infected cases, black-grayish nodules, about 1 to 2 mm. in diameter were found on the lung surface within 305 days of experiment already.

Upon observation of the lymph nodes, the tracheobronchial lymph nodes of ca. 5 to 7 mm. in diameter, black and firm, were seen in the cases
on the 423rd and the 523rd days of experiment. By the 608th, the 708th and the 802nd days, these nodules were found grown to 6 to 8 mm. in diameter.

Microscopic findings. In the early stage, coal dust particles were seen distributed diffusely throughout both the lungs (Fig. 1). On the 100th days, there was a plenty of dust particles lying free within the alveoli. In some areas, dust cells were collected into small aggregates but there was no evidence of fibrosis (Fig. 2). Upon 172nd days, nodular lesions with aggregation of coal dust particles were found in the sections of the lung. These nodules were observed consist of loose net-works of reticular fibers (Fig. 3). By 423th days, the dust lesions were found to have grown into the characteristic stellate form usually found in the lungs of colliers. In addition, these dust foci were each surrounded by focal emphysemata, so that the resemblance to the lesions in the colliers' lungs is further enhanced (Fig. 4 and 5). The reticulin fibres in these lesions were thicker and more compact.

On the 523th days, the stellate dust macule of the coal dust became more pronounced in the sections, in some places these dust macules having grown thicker and the centre of these stellate nodules became acellular and hyaline degeneration (Fig. 6 and 7).

The lesions, as found on the 608th days, were well-formed round nodules. This appearance of progressive fibrosis was particularly noticeable in the subpleural areas. These nodules were rather acellular and retinulin fibres had replaced collagen fibres (Fig. 8 and 9). Near the end of the 708 days' experiment, these nodules became confluent and the fibrosis advanced steadily, and in 802 days from the beginning of the experiment, the enhanced fibrotic nodules having confluent, acellular, collagen fibres and hyalin degeneration were found, mainly in the subpleural areas of the upper part of the lungs (Fig. 10, 11 and 12).

In the course of this experiment, the lung tissues sampled from two rats that died on the 172nd and the 305th days respectively were observed the influence of the bacteriological factor. These nodules were larger and round compared with the non-infected dust macules. The large nodules, ca. 1.5 mm. in diameter were found in the sections of the lungs on the 305th days. These infected nodules were histologically observed in many variations. This nodules were composed of numerous giant cells and dust cells with coal dust particles. Outside these nodules there were epitheloid cells, and lymphoid cells and in their central areas severe necrosis was observed. So that this nodules were like the tuberculous feature. These infected nodules showed also many reticulin fibres (Fig. 13 and 14).

In the tracheobronchial lymph nodules only many drifts of coal dust were observed in the early stage. Within 305 days of the experiment, the
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histological findings of the nodules were not as those of the fibrosis in the lung tissues. On the 523rd days, these lymph nodules were found to have fused together and formed enlarged cuboid nodules, containing thick and closely packed reticulin fibres. In some areas of the nodules, reticulin fibres had replaced collagen fibres. The progress is illustrated in Figs. 15, 16, 17 and 18.

Discussion

It is apparent from the foregoing results that the silicotic nodules can be produced in the lungs of rats by inhalation of mere coal dust without the intervention of rock dust, and it may be reasonably inferred that the inhalation of the simple coal dust can able to cause silicotic nodules in the lung tissue of rat.

On these experimental findings, the important thing is that the same stellate dust-macules as found in the lungs of colliers were produced in the lungs of this experimental animals, and silicotic fibrosis developed from these stellate dust foci. Moreover, the severe focal emphysemata produced around these stellate dust foci are also in close resemblance to such lesions in the anthracotic lungs of coal-workers. In short, the author has succeeded in obtaining lesions quite like the lesions in the lungs of silicotic colliers from this experimental anthracosis induced in rat’s lung by inhalation of the simple coal dust.

In the past, most investigators on this subject have opined that the inhalation of mere coal dust is not effective in producing massive fibrosis, not to speak of silicotic fibrosis. So, the typical silicosis frequent in coal-miners was attributed to the effect of the inhaled dust of siliceous rocks always present in coal pits. Concerning the histological findings of the anthracotic lungs of coal miners, the pathogenic effect of coal dust alone was seemed to produce the stellate form stadium of fibrosis at the limit, all the stadia beyond that being attributed to the effect of rock dust and not of the coal dust.

The etiology of the massive fibrosis frequent among the coal miners, however, was not clearly established owing to the lack of adequate study on the pathogeneity of mere coal dust in the lung tissue. James7) (1954) believes that tuberculosis is an essential factor. Another view is that a primary exposure to coal dust and a subsequent exposure to high concentration of silica cause the disease.

It is true that coal dust always contains a small portion of free silica dust, for example the coal dust I used in my experiments contained 0.67% of it. Considering the severity of the fibrotic lesions observed in this experiments, it is quite unreasonable to incriminate this small content of silica as the prime causative factor causing such a massive silicosis. The
quantity of free silica contained in coal dust seems to be too small to cause silicotic fibrosis to such an extent. The author concludes therefore that silica is not responsible here and wishes to suggest that the causative factor must be the total coal dust itself containing a minute quantity of silica element.

It is needless, I think, to add that silica dust of rocks is mixed in the coal dust in a more significant proportion, the fibrosis would be more progressive.

**CONCLUSION**

1. It is possible to produce experimental fibrotic anthracosis in the lungs of rats by artificial inhalation of mere natural coal dust.
2. The fibrotic lesions due to coal dust progressed to the stadia of silicotic nodules with collagenous and hyaline degeneration.
3. Infective process in the course of the experimental anthracosis speeded up the formation of fibrosis.
4. From these results, we are led to conclude that coal dust itself is effective enough in causing silicotic changes in the lungs of experimental rats.

**References**

2) Evans, Colliery Engin., 1951, 28, 465, 474, 513.
Fig. 1. Survival term 62 days. Exposure 270 hours. H. E. ×100. The coal dust particles are seen slightly in the alveolar walls of lung from a rat which received powdered coal dust.

Fig. 2. Survival term 100 days. Exposure 372 hours. H. E. ×150. Note that focal accumulations of coal dust are localized to region of subpleural area and showing thickening of alveolar walls.

Fig. 3. Survival term 172 days. Exposure 726 hours. H. E. ×100. Showing nodular focal accumulations of coal dust cells with some loose reticulin fibers (grade 1 fibrosis).

Fig. 4. Survival term 423 days. Exposure 1156 hours. H. E. ×100. Showing scattered stellate dust foci of reticulin fibers and accumulations of coal dust particles (grade 2 fibrosis).

Fig. 5. Some case in Fig. 4. Silver stain. ×100. Note that reticulin fibers in the stellate dust macule are seen thickly and compactly.

Fig. 6. Survival term 523 days. Exposure 1156 hours. H. E. ×100. Showing scattered stellate dust foci with compact reticulin (grade 3 fibrosis). Note that each stellate foci have focal emphysemata.
Fig. 7. Some case in Fig. 6. Silver stain. ×120. Note that the centre area of this stellate foci are seen collagenous and hyaline degeneration (grade 4 fibrosis).

Fig. 8. Survival term 608 days. Exposure 1554 hours. H. E. ×100. Note that the centre of the stellate dust foci shows more prompt structure that of the case in Fig. 6. The features of this dust foci are resemble to round nodular form.

Fig. 9. Survival term 608 days. Exposure 1554 hours. H. E. ×100. Showing well formed round nodules with collagenous and hyaline degeneration.

Fig. 10. Survival term 708 days. Exposure 1554 hours. H. E. ×60. Showing confluent nodules of densely packed collagen (grade 4 and 5 fibrosis).

Fig. 11. Some case as in Fig. 10. Silver stain. ×60. Showing multiple silicotic nodules (grade 5).
Fig. 12. Survival term 802 days. Exposure 1554 hours. H. E. ×20. Showing crowded and multiple silicotic nodules of fully collagenous fibrosis (grade 5).

Fig. 13. (infective type). Survival term 172 days. Exposure 726 hours H. E. ×100. Showing round and confluent coal dust macule without taking the stellate form of dust macule.

Fig. 14. (infective type). Survival term 305 days. Exposure 958 hours. H. E. ×30. Showing a large round nodule having the aggregation of coal dust particles.

Fig. 15. (on the feature of the tracheolymph nodule). Survival term. 305 days. Exposure 958 hours. H. E. ×20. Showing only many drifts of coal dust particles in the lymph nodule without having reticulin fibers.

Fig. 16. (on the feature of the tracheolymph nodule). Survival term. 523 days. Exposure 1156 hours. Silver stain. ×20. Showing scattered accumulations of coal dust with compact reticulin fibers and densely collagen in the lymph nodule.
Fig. 17. (on the feature of the tracheolymph nodule). Survival term 608 days. Exposure 1554 hours. Silver stain. ×30. Showing dense and swollen reticulin fibers in medulla of tracheobronchial lymph node.

Fig. 18. (on the feature of the tracheolymph nodule). Survival term 708 days. Exposure 1554 hours. Silver stain. ×30. Showing many hyaline silicotic nodules in medulla of tracheobronchial lymph node. The dense and reticulin fibers are stained black by silver salts.