The Influence of X-Ray on Experimental Pneumoconiosis

I, On the Development of Fibrotic Nodules Produced by Inhalation of Quartz Dust

By

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INTRODUCTION

Although, inhalation of quartz dust is the prime cause in the pathogenesis of silicosis, it is also believed that the condition of lung tissue susceptible to the impingement of quartz dust is of some importance for the development of the disease.

For this reason, the author has studied the influence of factors other than dust inhalation itself, and had an opportunity of finding a marked development of fibrotic nodules in the quartz dust inhaled lungs successively exposed to X-ray irradiation.

It is generally known that the lung tissue heavily irradiated with X-ray sometimes shows fibrosis clinically, so that it does not appear unreasonable that an irradiation would influence its susceptibility to silicosis in similar way, but my experiments showed the influence to be beyond any past anticipations.

To verify my occasional findings mentioned above, I have made some systematic experiments on the subject, of which I will report the results hereunder.

EXPERIMENTAL

Methods

The experimental animals consisted of 72 rats including both male and female, aged about 3 months and about 180 g. in average body weight. They were divided into 3 groups of 24 each and subjected to the 3 modes of experiments as follows, respectively.

Groups A and B were made to inhale quartz dust in the same dust

Detailed Histological Findings on the Photograph.

Fig. 1. Hx. E. $\times 100$ Fig. 2. Hx. E. $\times 100$

Fig. 1. Rat lung 60 days of survival term, 39 days of inhalation, 6 times of irradiation of X-ray: showing some slight granuloma.

Fig. 2. Control rat lung against (Fig. 1) rat: showing almost normal lung appearance.

Fig. 3. Hx. H. $\times 100$ Fig. 4. Hx. E. $\times 100$.

Fig. 3. Rat lung 175 days of survival term, 67 days of inhalation, 8 times of irradiation: showing one large nodule with coarse, and compact reticulin and a few collagen fiber (Grade 2 fibrosis).

Fig. 4. Control rat against (Fig. 3), survival 175 days, inhalation 67 days; showing some small granuloma.

cabinet, and Groups A and C were X-ray irradiated under the same conditions.

1) Method of inhalation: Quartz of Japanese product, containing 95.5% free crystal silica, as determined by N.S. Talvite's method, was pulverized in a ball mill and the dust dried in a sterilizer kept at 120°C immediately before use. The animals were exposed to a high concentration of this dust of 11,000 to 14,000 particles per cc. of air. 80% of the particles were below 3 micron in diameter, particles over 5 microns being only rarely contained. The dust blow was given for three hours each on three days every week.

Fig. 5. Hx. E. $\times 100$ Fig. 6. Hx. E. $\times 100$

Fig. 5. Rat lung 197 days of survival, 84 days of inhalation, 14 times of irradiation: showing one large nodule with compact reticulin and almost collagen fiber (Grade 3,4).

Fig. 6. Control rat against (Fig. 5.), survival 197 days, 84 days of inhalation: showing somewhat large granulosa with slight reticulin fiber.

Fig. 7. Hx. E. $\times 100$. Fig. 8. Hx. E. $\times 100$.

Fig. 7. Rat lung 260 days of survival term, 87 days of inhalation, 15 times of irradiation: showing many great and confluent fibrosis with compact reticulin fiber and collagenous fiber. (Grade 3,4)

Fig. 8. Control rat against (Fig. 7) 260 days survival, 87 days of inhalation: showing many granulosa and somewhat large silicotic granulosa with slight reticulin fiber.

2) Conditions of X-ray irradiation: A roentgen tube of 140 KVP was used under a tube current of 3 mA and with a 0.3 mm. Cu and a 0.5 mm. Al filters, giving a dose-rate of 7.1 r/min. at a distance of 50 cm. The animals in Groups A and C were given 30 r each on the whole body once a week.

3) Pathological technique: Routine postmortem examinations were carried out when an animal died or was sacrificed. The lungs of a killed animal were gently distended by injecting about 10 ml. of 10% formal saline through the trachea which was exposed at the neck. Most of the

Fig. 9. Hx. E. $\times 100$. Fig. 10. Hx. E. $\times 100$.

Fig. 9. Rat lung 267 days of survival term, 87 days of inhalation, 16 times of irradiation : showing many great nodule and confluent fibrosis with compact and collagenous fiber. Grade (3.4.)

Fig. 10. Control rat against (Fig. 9) 267 days survival, 87 days of inhalation : showing many granulosm and somewhat large granulosm with slight reticulin fiber.

Fig. 11. Mallory-Azan. $\times 100$. Fig. 12. Hx. E. $\times 100$.

Fig. 11. Rat lung 267 days of survival term, 87 days of inhalation, 16 times of irradiation : showing many great nodule and confluent fibrosis with compact and collagenous fiber (Grade 3. 4.)

Fig. 12. Rat lung 366 days of survival, 84 days of inhalation quartz only treated : showing somewhat great nodule with compact reticulin fiber and slight collagenous fiber.

air in the lungs was driven out by a gentle pressure on the sides of the thorax. The trachea was ligatured, the thoracic cavity was opened and the lungs, together with the subligated portion of the trachea, were taken out and fixed in formol. The lungs of the animals that died during the experimentation were first removed intact and then gently distended to the normal size, as satisfactory specimens could not be obtained otherwise. After for four days blocks were selected in the usual manner, cut into sections and stained with hamatoxylin-eosin and Mallory-Azan or Bielschowsky stains.

Gross Appearance of the Lungs.

Fig. 13. a: Rat lungs, control to b rat, 175 days of survival term, 67 days of inhalation quartz only.

b: Rat lungs 175 days of survival term, 67 days of inhalation, 8 times of irradiation.

Fig. 14. a: Rat lungs, control to b rat, 197 days of survival, 84 days of inhalation quartz only.

b: Rat lungs 197 days of survival term, 84 days of inhalation, 14 times of irradiation.

Fig. 15. a: Rat lungs 366 days of survival term, 84 days of inhalation quartz only.

b: Rat lungs 267 days of survival term, 84 days of inhalation, 16 times of irradiation.

Gross Appearance of the Lungs (2)

Fig. 16. a: Rats lungs in the A group; inhales quartz dust over five months, irradiated with 8 times or 15 times.

b: Rats lungs in the B group; inhaled quartz dust only over five months.

Results

The pathological changes produced in the lungs of the animals have been assessed according to Belt and King's classification. The five following grades of fibrosis were assumed: Grade 1, loose reticulin fibres with or without collagen formation; Grade 2, compact reticulin fibres with or without collagen formation; Grade 3, slightly cellular but almost collagenous; Grade 4, thoroughly collagenous and cellular, and Grade 5, acellular, collagenous and confluent.

1) Macroscopic findings of the lungs; In the first four months, the lungs and the pleura of the animals in all the groups shows little difference but in the fifth or sixth month, a great change came on in the lungs of every rat in the Group A, consisting in many nodular grayish-white spots of about 1 to 2 mm. in diameter on the surface of the lungs, mainly on the pos-

terior side. The lungs of these animals were also larger in general than of those in Group B.

In seven and eight months, these lesions observed in the Group A reached the maximum severity, but no evident change was found in any organ outside the lungs.

On the other hand, in the animals in the Groups B and C scarcely any change was obvious on the surface of the lungs.

2) Microscopic findings: By two months, marked changes had been produced in the lungs of the three sacrificed animals of the Group A, where multiple granulomata were recognized without individual reference, while in animals belonging to B, only one had a few granulomata in localized areas of its lungs.

In five months when other three animals of the each group were sacrificed, in the Group A, exposed to quartz dust on 84 days and to X-ray irradiation 14 times during the five months, the progress of silicotic fibrosis was found to have been very rapid indeed. Their lungs showed always slight or almost collagenous lesions of diffuse character (Grade 3) extending over wide areas, but the tracheobronchial lymph nodes were not yet very firm nor enlarged.

On the other hand, in the animals belonging to the Group B, we found only small fibrotic nodules of lower grade in some local fields of their lungs.

By the eighth or ninth month, four animals in the Group A subjected to more than 15 rounds of irradiation showed much more advanced fibrotic changes, severe fibrosis being found diffused all over the lungs in all cases with fibrotic nodules turned almost entirely collagenous (Grade 4).

In the Group B, made to inhale dust in the same manner as the Group A but not given X-ray irradiation, there were some fibrotic changes usually expected in such cases but no symptoms of packed reticulin fibres in their lungs, while in the Group C exposed to 15 rounds of X-ray irradiation but to no dust inhalation, no fibrotic change was ever found in their lungs.

3) Microscopic findings of other organs: No notable change was observable in the livers, the spleens and the kidneys of any animal within the eight months under observation, and there was no conspicuous change in the blood picture either.

DISCUSSION

By these experiments, I could ascertain that silicotic nodules were clearly developed in the Group A of animals exposed both to quartz dust inhalation and X-ray irradiation within 5 or 6 months, while in the Group C subjected only to X-ray irradiation, no obvious change in the pulmonary tissue was observed even in the tenth month. The animals in the Group A showed clear indication of fibrotic nodules within two months already,

and the development of such nodules in this group was so rapid that the Groups A and B became much differentiated in their silicotic changes thereafter.

Thus I succeeded in establishing my occasional finding of an increased and accelerated formation of silicotic nodules in the lungs of rats exposed to quartz dust inhalation and to X-ray irradiation to be not a mere occasional finding but an observation of a constant actuality.

It is generally known that X-ray irradiation acts in stimulating increased production of connective tissue, but clinically the increase is substantiated when the dose of irradiation is large enough, small doses hardly showing any appreciable effect.

In experimental works, however, some apparent increase of connective tissue cells following a small dosis of X-ray irradiation upon experimental irradiation have been reported by Hirose, Serizawa and Taguchi.

When heavy irradiation is administered, some fibrotic changes, such as cancers of the lungs and the breasts, have been reported to develop in the lung tissues, but when the dosis of the X-ray irradiation is small, for example, as in X-ray therapy of pulmonary tuberculosis, increase of fibrotic changes, though anticipated, have not been confirmed.

Nevertheless, it is quite rational to presume that the lung tissue should respond against a slight irradiation in much the same manner, at least in principle though not in degree, as against a heavy one.

Thus, it may be said that the strong response of the quartz dusted and X-ray irradiated lungs of rats represents a phase of the general rule of roentgen induced fibrosis, only somewhat accentuated by the combination of fibrotic tendencies induced by repeated quartz inhalation.

It is, so to speak, a misfortune that in silicosis the increase of the connective tissue means a progress of the disease, while in many other diseases, the same process means rather a cure.

CONCLUSION

1. Experimental silicosis was rapidly and intensively developed by quartz inhalation associated with simultaneous X-ray irradiation.
2. Mere quartz dust inhalation without accompanying X-ray irradiation induces silicotic nodules only slowly and moderately, in comparison with the preceding.
3. In animals exposed to X-ray irradiation only and to no dust inhalation, no evident changes were detected in the lungs within nine or ten months.
4. From these results mentioned, we might conclude that the X-ray, when applied to silicotic lungs, emphasizes the progress of the disease.