THE EFFECT OF GAMMA-RAYS (^{60}Co) AND ENDOXAN (CYCLOPHOSPHAMIDE) INJECTIONS ON SILICOTIC FIBROGENESIS IN RATS*

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Many hypotheses have been advanced to explain the pathogenesis of silicotic fibrosis. During the past 40 years two theories have mainly dominated silicosis research, namely the silica solubility theory[1] and the immunological theory.[2-7] Neither of these theories is at present accepted by all investigators of pneumoconiosis.

According to Vigliani and Pernis[7] the silicotic hyaline tissue has an immunological origin and corresponds to an antigen-antibody precipitate. It is known that circulating antibodies belong mostly to the gammaglobulins. Consequently the increased gammaglobulin fraction in the blood of silicotic patients[8] and of silicotic animals[9] might be the result of an increased antibody production or of an increase in gammaglobulins parallel to an increased antibody production. On the other hand, however, it is also true that an increase in gammaglobulins in the blood is not necessarily associated with an immunological reaction.

In the present investigation an attempt was made to determine whether the increased globulins in the blood of silicotic animals were directly involved in the genesis of the silicotic lesions.

It was considered that, should silicotic fibrosis be the result of an antigen-antibody reaction, suppression of antibody production would have an inhibitory effect upon the development of the fibrosis.

Different experiments were consequently performed to investigate the influence of ^{60}Co radiation and Endoxan injections on the blood cells and gammaglobulins of rats. As silicotic fibrogenesis is a relatively slow process, the appropriate dose had to be selected very accurately to ensure that the majority of the experimental animals survived at least about 60 days. On the other hand, the dose had to be big enough to inhibit the production of antibodies.

MATERIALS AND METHODS

In the final experiment reported here, 30 young male albino rats (*Rattus norvegicus*) were injected intratracheally[10] with a sterilized fine silica suspension (50 mg. of dust/animal) and divided into 3 groups, A, B and C. Group A served as the control.

On the 4th day after the silica administration, the 10 animals comprising group B each received 8 mg. of Endoxan intraperitoneally, and this injection was repeated every 7th day. Endoxan tablets were dissolved in isotonic saline yielding a final concentration of 8 mg. Endoxan/ml. of suspension. One animal died on the 18th day, one on the 54th day and 2 on the 59th day. The surviving 6 animals were sacrificed on the 60th day. Each of these animals received a total of 8 Endoxan injections.

On the 4th day after the silica injection the 10 animals in group C received 500 r of gamma-rays from a ^{60}Co source over a period of 15 minutes. Special adjustable wire-mesh containers were constructed to immobilize the rats during the period of irradiation. The rats were arranged in such a way that a line drawn along their longitudinal axes completed a circle with a radius of exactly 40 cm. from the ^{60}Co source (Fig. 1). The dose of irradiation is expressed as the number of roentgens per unit of time delivered at a distance of exactly 40 cm. from the source.

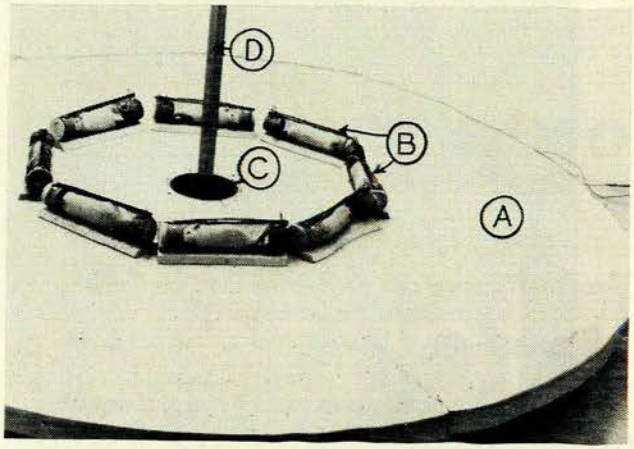

Fig. 1. Arrangement of rats during irradiation. A = table; B = containers with rats; C = opening leading to lead chamber; D = rod to which ^{60}Co source is attached.

After 7½ minutes, i.e. exactly half of the exposure time, the ^{60}Co source was quickly lowered into the lead chamber and the rats were rotated through 180° to ensure an even distribution of the dose over the body surface. Thirty days later the rats received a similar dose. One animal died on the 50th day and another 6 days later. The surviving animals, together with 6 of the control group (A), were sacrificed on the 60th day.

The histopathology of the lungs, the blood picture and the serum proteins of all these animals were studied. The lungs were fixed in 10% neutral formol saline and suitable blocks selected along their long axes at the level of the hilum. Serial sections were made at 6μ and were stained with haematoxylin and eosin and by silver impregnation.[11]

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The histological classification of the grade and the total amount of pulmonary fibrosis was done according to the method of Ross *et al.*;[12] total serum proteins were determined according the method of Weichselbaum ;[13] and cellulose acetate electrophoresis of the serum proteins was assessed as described by Kohn[14] and Scherr.[15]

RESULTS AND DISCUSSION

The results summarized in Table I indicate very clearly that the gammaglobulins, lymphocyte percentage and total

TABLE I. AVERAGE RESULTS OF 3 GROUPS OF ANIMALS*

	Group A: silica (*60 days*)	*Group B: silica + Endoxan* (*60 days*)	*Group C: silica + radiation* (*60 days*)
Average grade of fibrosis	2·8	3·0	3·1
Total fibrosis	8·0	10·2	10·7
Albumin	41·03	27·48	33·38
α_1-globulin	8·22	17·50	13·43
α_2-globulin	4·98	13·48	10·47
β_1-globulin	5·18	10·12	6·95
β_2-globulin	30·35	25·95	28·77
γ-globulin	10·23	5·47	7·00
Total protein	6·51	5·58	5·61
White cell count	7,917	23,600	2,233
Differential white cell count			
% neutrophils	35·17	64·67	46·83
% lymphocytes	57·17	31·50	48·17
% monocytes	2·33	1·33	1·83
% eosinophils	5·33	2·50	3·16

* Each value represents the mean of 6 animals.
Grades of fibrosis: grade I = loose reticulin fibres with no collagen; grade II = compact reticulin with or without a little collagen; grade III = somewhat cellular, but made up mostly of collagen; grade IV = wholly composed of collagen fibres and completely acellular; grade V = acellular, collagenous and confluent.

serum proteins of the experimental groups are significantly lower than those of the control animals. In spite of these results, the average grade and total amount of pulmonary fibrosis tend to be higher in the experimental animals. This is a rather surprising result, because it is contradictory to what one could expect if silicosis were the result of an antigen-antibody reaction. In addition, this finding does not support the statement by Vigliani *et al.*[8] that a rise in the gammaglobulins of silicotics should serve as an indication of active silicotic fibrogenesis in the lungs.

Careful microscopic examination of the lung sections shows that the experimental lungs contain fewer intact cells than the control sections. This observation is especially characteristic of the peribronchiolar lymphoid tissue which, in the experimental lungs, is almost free of intact lymphocytes. In these animals the circulating lymphocyte percentage is also lower than in the control animals.

The fibrosis of the experimental lungs is more diffuse, i.e. less nodular than that of the control animals. This difference may be ascribed to the fact that the experimental lungs contain fewer intact macrophages, with the result that the dust could not be effectively phagocytosed and concentrated.

The present experimental evidence indicates that neither the lymphocytes nor the circulating gammaglobulins are directly involved in silicotic fibrogenesis. The results therefore do not support an immunological pathogenesis for silicotic fibrosis, and are in accord with other experiments[16,17] carried out on the immunological theory.

SUMMARY

Albino rats were injected intratracheally with a fine silica suspension and divided into 3 groups, A, B and C. Group A served as the control. Group B received repeated Endoxan injections and group C was exposed to gamma-radiation (^{60}Co).

Injections of Endoxan and ^{60}Co radiation both cause a decrease in the total serum-protein concentration as well as a lowering of the albumin and gammaglobulin percentages. The circulating lymphocyte count and the cell population of the lungs of the Endoxan-treated and of the irradiated animals are consistently lower than those of the control animals. In spite of these changes, the total amount of pulmonary fibrosis is slightly higher in the irradiated and Endoxan-groups than in the control group.

The experiments show no positive correlation between the amount of pulmonary fibrosis and the concentration of gammaglobulins in the serum. The results therefore do not justify an immunological theory for the pathogenesis of silicotic fibrosis.

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