SILICA AND COLLAGEN IN THE LUNGS OF SILICOTIC RATS TREATED WITH CORTISONE

BY
B. D. STACY and E. J. KING

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The inhibiting effect of cortisone on proliferating connective tissue (Ragan, Howes, Plotz, Meyer, and Blunt, 1949; Spain, Molomut, and Haber, 1950a; Cavallero, Sala, Amira, and Borasi, 1951) and phagocytosis (Spain and others, 1950b) led us to observe the effect of this hormone on the developing silicotic nodule. Short-term studies on the effect of cortisone on the reaction of the mouse peritoneum to quartz showed a delay in macrophage response (Curran, 1952) and an inhibition of fibrosis (Magarey and Gough, 1952; Schiller, 1953). Over a longer period the effect of cortisone has been observed on the development of fibrosis in rats injected intratracheally with 50 mg. of quartz (Harrison, King, Dale, and Sichel, 1952). Cortisone treatment decreased the mobility of dust-laden phagocytes, and the marked alteration in the distribution of dust throughout the lung modified the normal pattern of fibrosis. The fibrosis in the cortisone-treated animals was irregular, diffuse, and generally less.

In this paper we report the analyses of lungs for silica and collagen, and hilar lymph glands for silica, from the above experiment on rats. The amount of collagen gave an indication of the amount of fibrosis; generally there was less in the cortisone group. The partition of silica between lung and lymph node gave a rough measure of macrophage activity. These analytical results confirmed previous histological findings.

This confirmation was not so marked when the same methods were applied to material from a later experiment in which King, Harrison, and Attygalle (1954) observed the effect of cortisone upon established silicosis. One hundred days after an intratracheal injection of quartz cortisone was given to a group of rats and the hormone treatment then continued regularly for 260 days. On histological examination the lungs of the cortisone-treated animals appeared similar to those of the controls, which had received quartz alone. The maximum grade of fibrosis was present in both groups 150 days after injection of the quartz, i.e., 50 days after the start of cortisone treatment, and this picture persisted till the end of the experiment. Chemical examination, however, showed differences between the two groups.

Material

The material analysed was taken from rats used in the experiments of Harrison and others (1952) and King and others (1954). All animals had been injected intratracheally with 50 mg. of quartz dust.

Methods

Lungs and lymph glands were bulked into groups as shown in the Tables. Silica was estimated by weighing the acid-insoluble residue of the ash. Collagen was estimated by determining the hydroxyproline content of lung hydrolysates, non-collagenous material being removed by precipitation with trichloroacetic acid (Janota, 1943; Harkness, 1952).

Preparation of Material.—Lymph glands were separated from the fixed lung, and lung and lymph glands were dried, bulked, and weighed. Remainders of blocks taken for sectioning were de-waxed and the dried tissue added to the groups. The bulked lungs were finally ground to a fine powder.

Silica Estimation.—A sample of material (containing about 10 mg. SiO₂) was weighed into a light (about 7 g.), hard glass centrifuge tube. The sample was ashed for 12 hours at 320° and then for 12 hours at 480°; 2N-HCl (3 ml.) was added to the ash, and the mixture warmed, with occasional stirring, on a boiling water bath for 30 minutes. After centrifuging, the extraction with warm 2N-HCl was repeated and the residue finally extracted by agitation with cold 2N-HCl. The residue (SiO₂) was weighed after drying at 105°. Recoveries of known similar amounts of quartz were in the range of 93 to 98%.
RESULTS IN CORTISONE-TREATED, SILICOTIC RATS

Collagen Estimation.—A sample (100 mg.) of dried ground lung was weighed into a thick-walled hard glass test tube graduated at 10 ml. Fat was removed by ether extraction, water (5 ml.) was added, and the mixture autoclaved for six hours at 30 lb. pressure. Water was added to 10 ml. and the tube heated for 15 minutes on a water bath. The mixture was made up to 10 ml., centrifuged and filtered. To 5 ml. of filtrate was added an equal volume of 10% trichloracetic acid and the mixture filtered after standing for one hour. Then 5 ml. of filtrate was transferred to a 50 ml. beaker and evaporated almost to dryness on a water bath. The residue was taken up in 6N-HCl (2 ml.) and washed with acid (4 ml.) into a hard glass test tube. The tube was sealed and autoclaved for four hours at 40 lb. pressure. The contents of the tube were made up to 10 ml. with 6N–HCl, shaken with charcoal, and filtered. A sample of this hydrolysate, after neutralization with 6N–NaOH and further appropriate dilution with a neutral mixture of 6N–NaOH and 6N–HCl, was used for the estimation of hydroxyproline following the method outlined by Neuman and Logan (1950a, b).

Results

Nature of Reticulin and Lung Collagen.—Some information as to the nature of reticulin and lung collagen was obtained using the standardized technique of two-dimensional filter paper chromatography. Reticulin was isolated from human renal cortex (Randall, 1953). Eighty per cent. of the dried material dissolved on autoclaving for six hours at 30 lb. The solution was hydrolysed with 6N–HCl. The amino-acid compositions of the reticulin hydrolysate and of a rat lung collagen hydrolysate were compared chromatographically with the hydrolysate of a calculated amount of purified commercial gelatin prepared from skin collagen. There was good correspondence between the position and intensity of spots. Glycine, hydroxyproline, and proline, the major constituents of "standard" collagen, were prominent in all cases. An outline of the amino-acid patterns is shown in Fig. 1. Hydroxyproline, which gave a faint yellow-brown spot with ninhydrin, was more easily compared by the highly characteristic pink spot which appeared after treatment with isatin followed by Ehrlich's reagent (Jepson and Smith, 1953). The chromatographic evidence suggested that reticulin and lung collagen resembled the more widely known collagens (Tomlin, 1953).

![Diagram of amino-acid composition](image-url)

Fig. 1.—The amino-acid composition of lung and skin collagen and of reticulin. Outline of amino-acids in two-dimensional filter paper chromatograms of acid hydrolysates of lung collagen, gelatin from skin collagen, and reticulin. Hydroxyproline detected by the method of Jepson and Smith (1953).
EFFECT OF CORTISONE ON THE MOVEMENT OF QUARTZ DUST FROM LUNGS TO LYMPH GLANDS IN DEVELOPING SILICOSIS

<table>
<thead>
<tr>
<th>Duration of Experiment (days)</th>
<th>No. of Rats in Group</th>
<th>Weight of SiO₂ (mg/rat) in</th>
<th>% SiO₂ in Dried Lymph Glands</th>
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<tr>
<td></td>
<td></td>
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<td>Cortisone</td>
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<td>4</td>
<td>27</td>
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<tr>
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<td>23</td>
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</table>

Cortisone treatment begun at 100 days.

**TABLE 2**

EFFECT OF CORTISONE ON DEVELOPING SILICOSIS

<table>
<thead>
<tr>
<th>Duration of Experiment (days)</th>
<th>No. of Rats in Group</th>
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*Individual lungs (sections photographed in original experiment), otherwise in bulked lungs.

Cortisone and Developing Silicosis.—This was studied first in the lymph glands and secondly in the lungs.

**Lymph Glands.**—Nodes from the controls were larger (Fig. 2a) and contained more quartz dust (Table 1 and Fig. 2b) than the "cortisone" nodes.

**Silica Content in Lung.**—The difference in silica content of the lungs from the two groups (Table 1) was not as marked as in the case of the lymph glands. Nevertheless, the cortisone lungs tended to contain more dust than the controls.

**Collagen Content in Lung.**—Collagen estimations were carried out on the bulked groups of some lungs, and on those individual lungs, sections of which had been photographed in the original experiment (Harrison and others, 1952). The results (Table 2) indicated that in the later stages of the experiment more lung collagen was present in the control than in the cortisone-treated group.

**Cortisone and Established Silicosis.**—The distribution of collagen and silica tended to follow the same pattern as in the experiment on developing silicosis. Lymph nodes from the controls were larger and contained more silica (Table 3) than the "cortisone" nodes. Control lungs contained correspondingly smaller amounts of silica. Of the

**TABLE 3**

EFFECT OF CORTISONE ON ESTABLISHED SILICOSIS: MOVEMENT OF QUARTZ DUST FROM LUNGS TO LYMPH GLANDS AND COLLAGEN CONTENT OF LUNGS

<table>
<thead>
<tr>
<th>Duration of Experiment (days)</th>
<th>No. of Rats in Group</th>
<th>Weight of SiO₂ (mg/rat) in</th>
<th>Lung Collagen</th>
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</thead>
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<tr>
<td></td>
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<td>Lungs</td>
<td>Lymph Glands</td>
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<td>Control</td>
<td>Cortisone</td>
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<td>3</td>
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</tr>
<tr>
<td>365</td>
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</tr>
</tbody>
</table>

*Cortisone treatment begun at 100 days.
three batches of lungs analysed in each group, the first two in the control series contained more total collagen than those in the cortisone series.

Discussion

Cortisone and Developing Silicosis.—Fig. 2 shows the weights and silica contents of lymph glands from both groups plotted against time. The relatively small amount of silica in the cortisone lymph nodes provides convincing evidence that continued administration of the hormone has interfered with the mechanism normally responsible for the transport of dust particles from the lungs to the lymph nodes. This is also shown by the results in Fig. 3 where the cortisone lungs are seen to contain more silica than the controls.

Although there was much variation in the size of the lymph glands, the percentage of silica in the glands, in all cases, was fairly constant (Table 1).

Table 1 shows figures of total silica content and its distribution between lung and lymph glands. There is little difference between the cortisone and control groups for the totals; in fact, they are roughly the same in the two groups and remain surprisingly constant over the course of the experiment. The amount of silica originally given intratracheally to each animal was 50 mg., and about 50% of this was found in the respiratory system after the first month. In the cortisone series nearly all the silica remained in the lungs, whereas in the control group about 30% was transported to the lymph nodes after 200 days. (A control animal with lymph nodes of exceptional size was analysed.
separately: over 50% of the retained silica appeared in the lymph nodes.

The results of the collagen estimations in the lungs show little difference between the two groups in the early phases of the experiment. After 150 days a difference appears (see Fig. 4), and the cortisone group has much less collagen than the control group. A similar impression was obtained histologically (Harrison and others, 1952), although decisive comparisons were difficult to make in some cases because of the diffuse, irregular pattern of fibrosis in the cortisone-treated animals.

![Diagram](http://oem.bmj.com/)

**Fig. 5.—Rate of accumulation of SiO₂ in the lymph glands.** Figures in brackets represent the number of rats in each group, heights of the blocks (underlined figures) represent the average mg. of SiO₂ in the lymph glands (figures in italics show the average dry weights of the lymph glands). A. Cortisone and developing silicosis; B. cortisone and established silicosis; C. untreated silicosis (quartz control).
RESULTS IN CORTISONE-TREATED, SILICOTIC RATS

A combination of chemical and histological methods for assessing fibrosis has also been recently reported by Aterman (1954). Estimating collagen by the chemical method described above, he found a satisfactory correlation between histological and chemical results in studying fibrosis produced in the liver by carbon tetrachloride. In these experiments, too, cortisone was found, at certain stages, to inhibit the formation of collagen.

Cortisone and Established Silicosis.—In the control animals there was, over the first 200 days following the injection of quartz, an almost linear increase with time in the amount of silica in the lymph nodes, indicating continuous regular phagocytic activity (Fig. 2b). If cortisone was given (see Table 3), this activity was depressed, and there was a little increase of silica in the lymph nodes after the start of cortisone treatment. Indeed, further deposition of dust in the lymph glands was effectively inhibited throughout the whole period of cortisone administration. From tissue culture work, Paff and Stewart (1953) have made the general observation that "cortisone can effectively block the nomadic proclivities of the free wandering cell" (macrophage)—a conclusion which would seem to apply equally well to the above results.

As to fibrosis, it has been found histologically that the two groups are qualitatively similar (King and others, 1954), yet on a chemical basis the lungs of the controls tend to contain more collagen (Table 3). This discrepancy reflects the inadequacy of the histological method for measuring fibrosis quantitatively. Beyond the visual assessment, which at five months showed the maximum grade of fibrosis in both groups, the histological method could throw little light on further collagen formation. But the quantitative chemical estimation detects this further development, and thus yields results that show a difference between the two groups.

Studies in vivo (Wolbach, 1933) and in vitro (Porter and Vanamee, 1949; Porter, 1951; Gerarde and Jones, 1953) have led to the claim that collagen is a product of the metabolic activity of fibroblasts. The lower collagen values found in the treated group lend support to the thesis that cortisone modifies fibroblast activity.

Lymph gland results from both experiments are combined in Fig. 5. The heights of the blocks in this diagram represent lymph gland silica (mg. per rat); the average dry weights of glands and the number of animals in each block are also shown. That cortisone has exerted a marked effect on the dust content of lymph glands in both experiments is clearly demonstrated in this diagram.

Summary

The lungs of cortisone-treated and control silicotic rats have been analysed for silica and collagen, and the lymph glands for silica. The experiments tested the effect of cortisone on (a) developing and (b) established experimental silicosis.

In both experiments cortisone (a) decreased the transport of quartz dust from the lungs to the hilar lymph nodes, and (b) inhibited the formation of collagen in the lungs.

The results have been correlated with previous histological findings.

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